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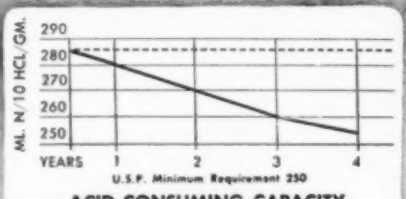

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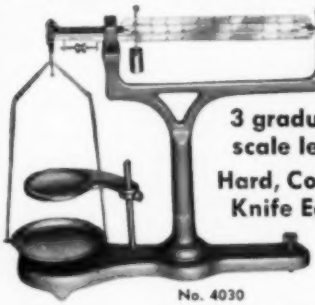

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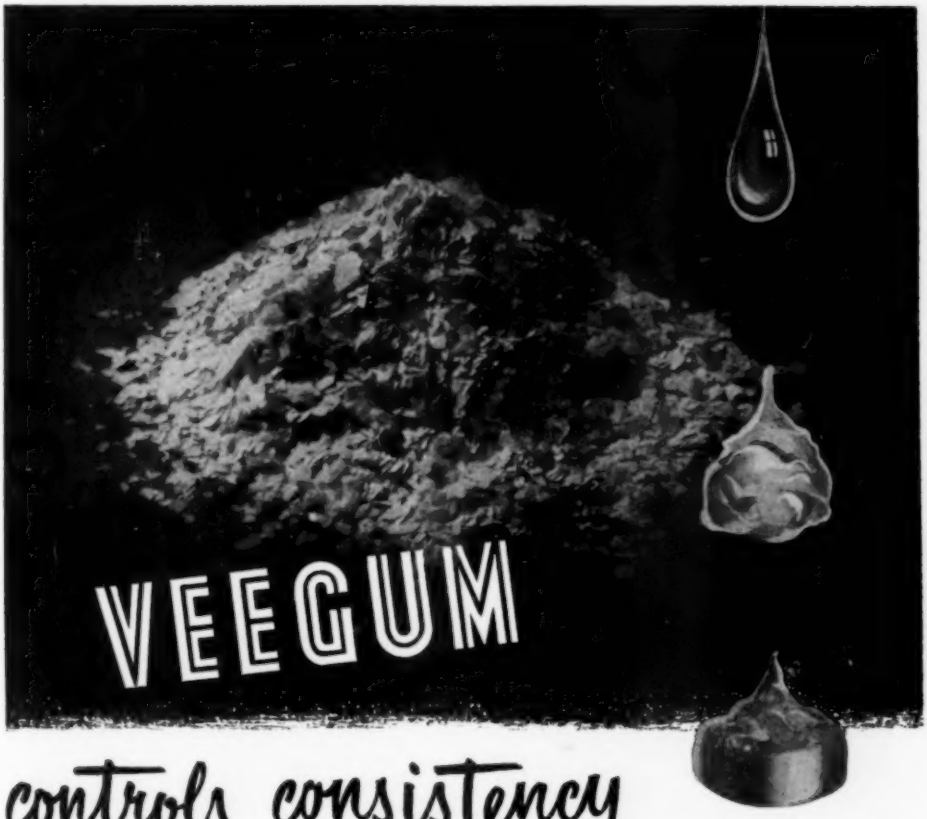

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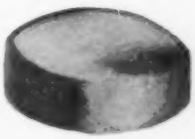

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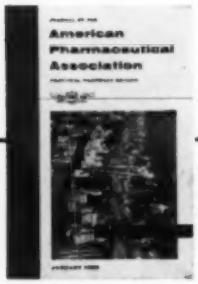

A Preview of Advertising

In the August 1956 issue of the Practical Pharmacy Edition

(All references to actions and uses are taken from the statements of the producers of the products mentioned.)

▸ In its customary position on the inside front cover **Abbott Laboratories** answers the question of what is a good B complex in its announcement of **Sur-Bex** *with* **C**—its vitamin supplement, recommended in cases of dietary deficiencies.

▸ "*The Druggists' Own*" is the familiar phrase that has long identified **The American Druggists' Insurance Co.** which emphasizes in its current announcement its strength, safety and security. The advertiser notes that it also makes protection available for the home and to employed pharmacists, suggesting that one of its agents be consulted for complete details.

▸ **Lutrexin Tablets,** product of **Hynson, Westcott & Dunning,** are reported to have relieved, in many cases, "the entire symptom complex of dysmenorrhea."

▸ In its announcement of **Multicebrin, Eli Lilly and Company** points out that an extensive advertising and detailing program is supporting the promotion of this multiple-vitamin product and asks the logical question: "Is *your* stock adequate?"

▸ An important name change is noted in the announcement that on August 1, 1956, **Sharp & Dohme,** the pharmaceutical and biological division of **Merck & Co., Inc.,** adopted the name **"Merck Sharp & Dohme"** and a new trademark "to reflect the teamwork which has already produced significant new medical products."

▸ A light (or perhaps we should say, as the advertiser does, "sunny") note is introduced in the **Parke, Davis & Company** announcement of **Caladryl,** indicated as useful in treating sunburn, prickly heat, poison ivy, insect bites and minor skin irritations.

▸ Marking a dramatic "first", **Schering Corporation** has, for the first time in THIS JOURNAL, employed a four-color two-page insert to point up graphically its **Meticorten** and **Meticortelone.**

▸ **Smith, Kline & French Laboratories** offers further testimony, in the person of an active pharmacist-retailer, of the effectiveness of its *display carton* in promoting the sale of **Benzedrex Inhalers.**

▸ **The Upjohn Company's** announcement is a timely one about an established product for which the demand is particularly pressing at this time of the year—**Kaopectate**—for *diarrhea.*

▸ Described as "Agent of Choice in Vaginitis" is *new* **Sterisil** *vaginal gel,* offered by **Warner-Chilcott.**

▸ The **Winthrop Laboratories** announcement, in delineating the multiple uses of **pHisoHex,** demonstrates graphically some of the many situations wherein this detergent is proving useful in effecting "hospital clean" conditions.

Look for details in the Practical Pharmacy Edition of THIS JOURNAL—Out August 20

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLV — *AUGUST 1956* — NUMBER 8

CONSECUTIVE NO. 16

Determination of the Excretion and the Tissue Distribution of Carboxyl Carbon in the Rat after Administration of C^{14} Carboxyl Acetylsalicylic Acid*,[1]

By W. R. BORST, J. E. CHRISTIAN, and T. S. MIYA

To determine the excretion and the tissue distribution of C^{14} carboxyl acetylsalicylic acid in the rat, 450 mg./Kg. and 20 mg./Kg. dose levels containing 2 μc. of C^{14} per dose were administered by stomach tube. The tissue distribution was compared by using the tissue-plasma ratio per mg. dry and wet tissue weight. After forty-eight hours the urine excreted 83.3 per cent of the C^{14} and 9 per cent was found in the feces. No C^{14} was found in the expired carbon dioxide. When studied 30 minutes after administration kidney and lung tissue showed the highest accumulation of the C^{14} while the red blood cells and the brain showed the lowest.

FOR MORE THAN half a century acetylsalicylic acid, more commonly known as aspirin, has been used empirically in the treatment of headache, neuralgia, arthralgic pains, rheumatic fever, and grippal conditions. The analgetic effectiveness of the drug is well known to exist. As a pain killer, fever reducer and headache reliever, the mechanism of action, however, is not understood.

Lignon (1) showed that acetylsalicylic acid is absorbed from the intestinal tract unhydrolyzed by using intestinal loops. He found that the outflowing solution contained no more free salicylic acid than the acetylsalicylic acid solution introduced into the loop.

The relatively short effect of the analgetic action of acetylsalicylic acid is probably due to the enzymatic hydrolysis of the drug. Lester (2) has proposed a theory that the analgetic action of acetylsalicylic acid is exercised by the unhydrolyzed intact molecule in the plasma. Uhlenbroock (3) found that the intact acetylsalicylic acid molecule inhibited cell respiration very much less than did the salicylic acid molecule.

Mandel (4) using C^{14}-1 acetyl C^{14}-carboxyl salicylic acid found acetylsalicylic acid and salicylic acid in the plasma after oral administration and established that unhydrolyzed acetylsalicylic acid was in the plasma of humans for approximately 2 hours after administration of the drug. Knouse (5) using acetyl-1-C^{14} salicylic acid at a dose level of 200 mg./Kg. in the rat found that the greatest activity was in the intestine, muscle, blood, and kidney cortex. There was a low excretion of radioactivity in the urine and 40 per cent of the administered radioactivity was found in the expired carbon dioxide. The salicylate level was found to rise for forty minutes after oral administration of the drug and then level off while the radioactivity reached a maximum earlier.

It was also found that the radioactivity level decreased more rapidly than the salicylate level on intravenous administration of the drug.

* Received August 11, 1955, from the Bionucleonics Laboratory of the Purdue University, School of Pharmacy, West Lafayette, Ind.

[1] C^{14} was obtained on allocation from the U. S. Atomic Energy Commission.

From these results the acetate portion of the drug appears to be rapidly hydrolyzed from the aspirin molecule and follows different metabolic pathways than that found for the salicylate molecule.

The purpose of this work was to study the pathways to excretion of acetylsalicylic acid in the rat and to determine the degree of accumulation of the drug in certain organs 30 minutes after oral administration of a suspension of the drug.

EXPERIMENTAL

Determination of the Distribution of C^{14} in the Excretion Products.—C^{14} carboxyl acetylsalicylic acid, 450 mg./Kg., and containing a total of 2 μc. of C^{14} was administered through a stomach tube to each of a group of five white, female (Purdue-Wistar Strain) rats, the average weight of which was 200 Gm. The dose was suspended in 2 ml. of a 1% Methocel[2] solution.

The expired carbon dioxide was absorbed for periods of twelve, eighteen and eighteen hours (forty-eight hours total) in separate pairs of absorbers as described by Mackenzie (6). The absorbers were connected to a metabolism cage[3] similar to that of Roth (7) in which the urine and feces were collected separately over a period of forty-eight hours.

A Model D 46 gas flow counter, Nuclear Instrument and Chemical Company, was used to measure the radioactivity of the samples. The efficiency was found to be 40.8%, using a National Bureau of Standards C^{14} standard. Each tissue sample was counted for a sufficient time to give a relative standard error of 1.5% or less.

Suitable aliquots of the urine and feces homogenate were deposited on special planchets.[4] Aliquots of the expired air-absorber solutions to yield approximately 50 mg. of barium carbonate were used for precipitating the sodium carbonate as barium carbonate. The activity of these samples was measured in the gas flow counter. Duplicate samples were made.

Self absorption of the C^{14} in the samples was corrected by the method of Heard (8). Table I gives the results.

These results are in agreement with those reported by Alpen (9) in the human and dog, and Schayer (10) in the rat. Both of these authors used C^{14} carboxyl salicylic acid. This similarity of per cent of excretion products for both the acetylsalicylic acid and salicylic acid is in accord with the findings of Lester (2) and Smith (11) who showed that the metabolites excreted by the animal, from administered acetylsalicylic acid, are identical with those of administered salicylic acid found by Kapp (12).

Determination of the Tissue Distribution of C^{14}.—C^{14} carboxyl acetylsalicylic acid was administered to two similar groups of rats in the same manner as given under *Determination of the Distribution of C^{14} in the Excretion Products*. A dose of 450 mg./Kg. and also of 20 mg./Kg. of the acid containing a total of 2 μc. C^{14} in each dose was used.

Table I.—Radioactivity of Excretion Products Forty-Eight Hours After Oral Administration of C^{14} A.S.A.

Material	Total Activity,[a] dis./min.	Total Activity, %
Total radioactivity (5 doses)	2.22×10^7	100.0
Urine	1.85×10^7	83.3
Feces	0.2×10^7	9.0
Expired carbon dioxide	0.0	0.0

[a] Total radioactivity in disintegrations per minute of the pooled samples taken from 5 animals.

Thirty minutes after drug administration the animal was sacrificed by cervical dislocation. Most of the blood was removed by intracardial puncture, oxalated and centrifuged for obtaining the plasma and red blood corpuscles (R. B. C.). The liver, spleen, heart, brain, lung, kidney, adrenal and pituitary glands were removed, blotted dry, and weighed. The intestinal tract was removed for determining the nonabsorbed aspirin. The activity remaining in the carcass represented the absorbed drug other than that in the removed tissues.

Homogenates or solutions were made of each of the five combined organs, intestinal contents, and carcasses for radioactivity measurement. The homogenates were prepared in such volume that 0.2 ml. of homogenate gave approximately 10 mg. of dried residue. The plasma was deposited without further treatment while the R. B. C. were hemolyzed with distilled water.

The activity of the homogenates, solutions, and blood fractions was measured by the same counting setup used in the previous radioactivity measurements. Suitable aliquots of the liquids were deposited on the special planchets and dried at 50°.

Self absorption of C^{14} in the dried homogenate samples was corrected from an absorption correction curve plotted from data obtained from the liver homogenate samples containing known quantities of standard C^{14}. Self absorption in the blood fractions was corrected by the method of Heard (8).

The total activity per mg. of dry and wet tissue weight, together with the tissue-plasma ratios per mg. dry and wet tissue for both dose levels are given in Table II. The per cent of dry tissue weight was determined by drying an aliquot of the tissue as a homogenate to constant weight at 50°.

At the 450 mg./Kg. dose, 2.68×10^6 dis./min., equivalent to 12.1% of the total dose was absorbed into the animal body. At the 20 mg./Kg. dose 9.11×10^6 dis./min., equivalent to 41.3% was absorbed.

From the tissue-plasma ratio per mg. dry tissue for the accumulation of the drug at the higher dose level, the kidney showed 82% and the lung 63% accumulation of that found in the plasma. On the same basis the liver, heart, adrenal, and pituitary glands showed about 35% and the spleen and brain showed about 10% of the accumulation found in the plasma.

Similarly from the tissue-plasma ratio per mg. wet tissue for the accumulation of the drug at the higher dose level, the kidney showed 224%, adrenal glands 130%, and the lung 106% of that found in the plasma. On the same basis the liver and pitui-

[2] Methocel X-2602, Dow Chemical Company (3000 cps).
[3] Wakefield Industries Inc., 5108 W. Grove St., Skokie, Ill.
[4] The special planchets were prepared by depositing a 1/8" wide Dow Corning High Vacuum Silicone grease layer around the outside circumference of the planchets to give a center area of 5.31 cm.^{2}.

TABLE II.—RADIOACTIVITY OF TISSUES THIRTY MINUTES AFTER THE ADMINISTRATION OF C^{14}-ACETYLSALICYLIC ACID

Organ	Total Activity,[a] dis./min. per mg. Dry and Wet Tissue (450 mg./Kg. dose) Dry	Wet	Total Activity,[a] dis./min. per mg. Dry and Wet Tissue (20 mg./Kg. dose) Dry	Wet	Tissue-Plasma Ratio per mg. Dry and Wet Tissue 450 mg./Kg. dose Dry	Wet	20 mg./Kg. dose Dry	Wet
Liver	63.0	14.0	82.0	18.0	0.31	0.82	0.15	0.39
Spleen	20.0	4.9	45.0	11.0	0.10	0.29	0.08	0.24
Heart	61.0	10.0	111.0	26.0	0.31	0.62	0.20	0.56
Brain	19.0	4.9	13.0	3.5	0.10	0.29	0.02	0.08
Lung	125.0	18.0	220.0	28.0	0.63	1.06	0.41	0.61
Kidney	163.0	38.0	327.0	73.0	0.82	2.24	0.60	1.58
Adrenal	76.0	22.0	46.0	13.0	0.38	1.30	0.09	0.29
Pituitary	62.0	14.0	65.0	15.0	0.31	0.86	0.12	0.33
Plasma	200.0	17.0	542.0	46.0	1.00	1.00	1.00	1.00
R. B. C.	6.5	1.0	11.0	1.7	0.03	0.06	0.02	0.04
Carcass	9.7	2.0	49.0	9.9	0.05	0.12	0.09	0.22

[a] Total radioactivity in disintegrations per minute of the pooled samples taken from 5 animals. Average of duplicate determinations.

tary showed about 85%, the heart 62%, and the spleen and brain 29%.

From the tissue-plasma ratio per mg. dry tissue for the accumulation of the drug at the lower dose level, the kidney showed 60% and the lung 41% accumulation of that found in the plasma. On the same basis the liver and heart gave about 20%, the spleen, adrenal, and pituitary showed about 10% and the brain 2% of the amount found in the plasma. On the same basis the R. B. C. showed about 3% accumulation of the drug at both dosage levels.

Similarly from the tissue-plasma ratio per mg. wet tissue for the accumulation of the drug at the lower dose level, the kidney showed 158% of that found in the plasma. On the same basis the heart and lung showed about 60%, the liver and pituitary about 35%, the spleen and adrenal glands about 25%, and the brain 8% accumulation of the drug as that found in the plasma. On the same basis the R. B. C. showed about 5% accumulation of the drug at both dosage levels.

The adrenal glands and brain showed the greatest variation in the amount of drug accumulated when the two dose groups were compared. The lung and kidney showed the highest and the brain and red blood cells showed the lowest amount of accumulation of the drug at both the dosage levels (see Table II).

The unexpected variations in the results found in the case of the adrenal glands were checked by administering similar doses to each of 5 rats at both the 450 mg./Kg. and 20 mg./Kg. dose levels. The results found for the tissue-plasma ratio per mg. of dry adrenal tissue were 0.33 and 0.13 as compared to 0.38 and 0.09 previously found (see Table II) for the 450 mg./Kg. and 20 mg./Kg. dose levels, respectively.

The tissue-plasma ratios per mg. of wet adrenal tissue were 113 and 42 as compared to 130 and 29 previously found for the 450 mg./Kg. and 20 mg./Kg. dose levels, respectively.

The adrenal tissue appears to accumulate a higher amount of the carboxyl C^{14} when the total salicylate dose is larger.

At the higher dose 12.1% (10.9 mg. drug per 200 Gm. rat) of the drug was absorbed from the gastrointestinal tract in the thirty-minute period of which 63.2% of the absorbed drug was accounted for in the carcass. At the lower dose 41.3% (1.6 mg. drug per 200 Gm. rat) was absorbed and 78.5% of the absorbed drug was found in the carcass.

SUMMARY

The total radioactivity found in the excretion products after forty-eight hours metabolism of the drug was 92.3 per cent of which 83.3 per cent was found in the urine and 9.0 per cent in the fecal excretion with none found in the expired carbon dioxide.

Thirty minutes after the administration of 450 mg./Kg. and 20 mg./Kg. dose levels containing 2 μc. of C^{14} per dose per rat in two groups of five rats each, all the organs analyzed showed significantly high activity. The kidney and lung showed the highest and the red blood cells and the brain showed the lowest accumulation of the drug at both dosage levels.

At the higher dose 12.1 per cent of the drug administered was absorbed from the gastrointestinal tract in the thirty-minute period. Of the total drug absorbed 63.2 per cent was found in the carcass. At the lower dose 41.3 per cent was absorbed and 78.5 per cent of the absorbed drug was found in the carcass.

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Derivatives of Salicylamide*

By JOHN A. FAUST, LEONARD H. JULES, and MELVILLE SAHYUN

A series of fifty-four derivatives of salicylamide was prepared for evaluation as analgetics and/or antimicrobial agents. The methods of synthesis and some of the chemical properties of these compounds are described. The most active analgetic compound of the series was 2-allyloxybenzamide, the potency of which was found to be about three times that of salicylamide. Certain of the compounds, notably 3,5-diisopropylsalicylamide, exhibited appreciable fungicidal activity.

DURING THE COURSE of an investigation (1) dealing with the synthesis of 3-, 4-, and 5-phenylsalicylamides as possible analgetic and/or antimicrobial agents, a number of similar derivatives of salicylamide were prepared for comparison of their biologic activities with those of the corresponding phenyl-substituted compounds. The majority of the salicylamide derivatives prepared for these studies had not been described in the literature, and these compounds are the subject of this paper.

A detailed study of the analgetic activity of these congeners of salicylamide has been reported by Way, *et al* (2). According to these investigators, several of the compounds are more potent analgetics in the experimental animal than salicylamide with the highest activity being exhibited by 2-allyloxybenzamide (Compound S.2-347) which was about three times as potent as salicylamide. It is interesting to note that this compound has also been reported to possess narcotic activity (3).

Representative examples of the series were also studied by K. S. Pilcher[1] as antimicrobial agents, particularly against pathogenic organisms such as *Trichophyton mentagrophytes, Candida albicans, Actinomyces necrophorous,* and *Corynebacterium pyogenes.* Several compounds exhibited moderate activity against *Trichophyton in vitro,* the most potent being 3,5-diisopropylsalicylamide (Compound S.2-150) which was effective in a dilution of 1:64,000. The most active compounds against *Actinomyces* were 3,5-diisopropylsalicylamide (Compound S.2-150) and 5-bromo-N-cyclohexylsalicylamide (Compound S.2-358) which in dilutions of 1:32,000 inhibited the growth of these organisms both in liquid glycolate media with and without serum. None of the compounds in this series which were tested against *Candida* and *Corynebacterium* was found to have significant activity.

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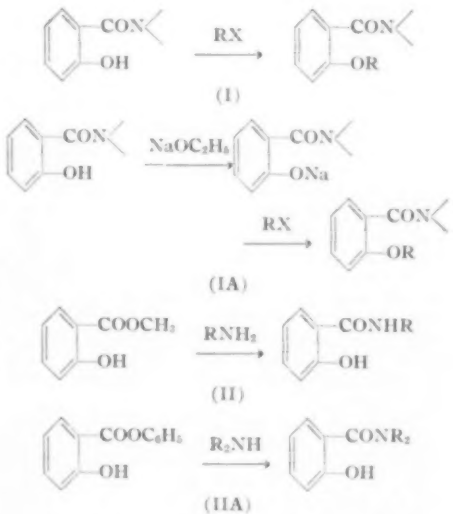
This investigation was supported by Cutter Laboratories, Berkeley, Calif.
[1] Private communication.

DISCUSSION

The majority of the compounds listed in the tables were prepared by one of the methods outlined below, and representative examples of each method are detailed in the Experimental Part.



R = alkyl or substituent as indicated in the tables.
X = halogen.

Alkylations of salicylamide derivatives with alkyl halides other than basic alkyl halides were carried out according to method I, using either anhydrous potassium carbonate or sodium hydroxide to provide the basic medium. The basic halide, 2-diethylaminoethyl chloride, was reacted with sodium salts of the salicylamides in substantially water- and alcohol-free toluene, a procedure which greatly diminished the tendency for the basic chloride to dimerize.

The migration of the allyl group of allyl ethers of phenol to form C-allyl isomers by heat is well known (4). This rearrangement has also been noted with allyl ethers of salicylic acid compounds (5). Many of the allyl ethers of salicylamide derivatives prepared during the present work were oils, and with one exception, were purified by vacuum distillation. Some isomerization occurred during distillation, as was indicated by a positive ferric chloride test for

phenols. The distillate was freed of these phenolic compounds by extraction with cold, dilute sodium hydroxide. In one instance (Compound S.2-435) the b. p. at 0.5 mm. was sufficiently high to cause almost completely the rearrangement to the phenolic isomer, and the compound could not be purified by distillation.

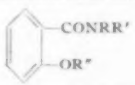
The reaction between an ester of the appropriate salicylic acid derivative and an amine was employed to prepare the various salicylamides as indicated in methods II and IIA. Ammonia and primary amines reacted smoothly with the readily available methyl salicylates, but secondary amines reacted very slowly or not at all. Secondary amines reacted with phenyl esters, however, and the N,N-disubstituted amides were obtained by this procedure which has been described previously (6).

Since these studies were completed three compounds listed in the tables have appeared in the literature: N,N-dimethylsalicylamide (Compound S.2-420) (7) and N-isopropylsalicylamide (Compound S.2-127) (8) have been studied as analgetics, and the chemistry of gentisamide (Compound S.2-212) has been recorded (9). All of the other compounds listed have not, as far as we are aware, appeared in the literature available to us. The yields given refer, in most instances, to pure products and no attempts were made to obtain maximum yields by varying the conditions under which the reactions were run. The analytical data were obtained by R. H. McCutcheon of these Laboratories.

EXPERIMENTAL

Intermediates.—Besides well-known esters and amides, the following intermediates used in these studies have been reported in the literature: N-methylsalicylamide (10), N,N-diethylsalicylamide (11), N-hydroxyethylsalicylamide (12), N-phenylsalicylamide (13), salicyloylurea (14), methyl 5-bromosalicylate (15), phenyl 5-bromosalicylate (16), 3,5-diisopropylsalicylic acid (17), methyl 3,5-diiodo-

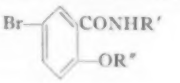
TABLE I.—DERIVATIVES OF SALICYLAMIDE



Code No. S.2-	R	R′	R″	Method of Prepn.	Yield, %	M. p., °C.	Recryst. Solvent	Nitrogen, % Calcd.	Found
126	H	$CH_2CH_2C_6H_5$	H	II	60	94–95	Dil. i-PrOH	5.80	5.81
127	H	$CH(CH_2)_2$[a]	H	II	33	66–67	Dil. EtOH	7.81	7.66
420	CH_3	CH_3	H	IIA	71	163–165	Acetone	8.48	8.37
315	C_6H_{11}[b]	C_6H_{11}[b]	H	IIA	11	193–194	EtOH	4.65	4.64
559	CH_3	$CH_2C_6H_5$	H	IIA	52	103–104	$CHCl_3$-heptane	5.81	5.70
42	H	H	CH_2CH_2OH	I	40	114–116	$CHCl_3$	7.73	7.54
120	H	H	$CH_2CH_2N(C_2H_5)_2$ HCl	IA	50	156–158 (Decompn.)	i-PrOH-ether	10.23	10.11
138	H	H	CH_2CH_2COOH	..[c]	24	175–176	Water	6.70	6.59
347	H	H	$CH_2CH{=}CH_2$[d]	I	85	97–98	.....[e]	7.90	7.78
404	H	H	$CH_2C_6H_5$	I	88	116–117	Dil. i-PrOH	6.21	6.25
407	H	H	$CH_2C{=}CH_2$	I	91	152–153	Water	8.00	8.04
507	H	H	$CH(CH_3)CH_2CH_3$	I	20	68–70[f]	.....	7.25	7.17
247	H	CH_3	CH_3	IA	33	Oil[g]	.....	8.48	8.36
436	H	C_2H_5	$CH_2CH{=}CH_2$	I	90	Oil[h]	.....	6.82	6.57
434	CH_3	CH_3	$CH_2CH{=}CH_2$	I	59	Oil[i]	.....	6.82	6.72
435	H	CH_2CH_2OH	$CH_2CH{=}CH_2$	I	89	Oil[j]	.....	6.33	6.23
220	H	$C_6HC_4OOH(p\text{-})$	$COCH_3$	..[c]	36	222–223 (Decompn.)	Dil. acetone	4.68	4.50
313	H	C_6H_{11}[b]	$CH_2CH(OH)CH_2OH$	I	58	110–112	$CHCl_3$-heptane	4.77	4.74
314	H	C_6H_{11}[b]	$CH_2CH_2N(C_2H_5)_2$ HCl	IA	50	125–128	i-PrOH-ether	7.89	7.79
316	H	C_6H_{11}[b]	$CH_2CH{=}CH_2$	I	62	79–80	Dil. EtOH	5.40	5.39
326	H	C_6H_{11}[b]	$CH_2CH(Br)CH_2Br$	..[c]	75	95–96	Dil. i-PrOH	3.34	3.36
364	H	C_6H_{11}[b]	CH_2COOH	I	56	144–145	Dil. MeOH	5.05	5.20
366	H	C_6H_{11}[b]	$CH_2COOCH_2CH_2N(C_2H_5)_2$·HCl	..[c]	62	171–172	i-PrOH-ether	6.78	6.78
525	H	OH	$CH_2CH{=}CH_2$	II	73	129–130	Water	7.25	7.08
521	H	NH_2 HCl	$CH_2CH{=}CH_2$	II	63	171–172	i-PrOH	..[k]	..
519	H	$CONH_2$	$CH_2CH_2CH_2CH_3$	I	14	170–171	.....[l]	11.86	11.61
513	H	$CONH_2$	$CH_2CH{=}CH_2$	I	34	186–187	Acetone	12.72	12.65
421	CH_3	CH_3	CH_2CH_2OH	I	66	Oil[m]	.....	6.69	6.62
558	CH_3	CH_3	$CH_2CH_2N(C_2H_5)_2$·CH_3I	I	64	148–150	EtOH-EtAc	6.89	6.59
419	C_2H_5	C_2H_5	CH_2CH_2OH	I	59	Oil[n]	.....	5.90	6.07
437	C_2H_5	C_2H_5	$CH_2CH{=}CH_2$	I	87	Oil[o]	.....	6.00	5.95
587	C_6H_{11}[b]	C_6H_{11}[b]	$CH_2CH_2N(C_2H_5)_2(CH_2)_2SO_3$	IA	68	190–192	i-PrOH-ether	5.32	5.30

[a] Ref. (8). [b] C_6H_{11} = Cyclohexyl. [c] See Experimental Part. [d] Ref. (3). [e] Not recrystallized but precipitated from an alcohol solution by the addition of water. [f] B. p., 136–139° (1 mm.). The distillate solidified and was not recrystallized. [g] B. p., 127–129° (1 mm.). [h] B. p., 130–133° (1 mm.). [i] B. p., 126–128° (1 mm.). [j] This compound rearranges during distillation at 0.5 mm. [k] Chloride analysis: Calcd., 15.51; Fd., 15.38. [l] Not recrystallized. [m] B. p., 160–162° (0.5 mm.). [n] B. p., 160–162° (1 mm.). [o] B. p., 127–130° (1 mm.).

TABLE II.—DERIVATIVES OF 5-BROMOSALICYLAMIDE



Code No. S.2-	R'	R"	Method of Prepn.	Yield, %	M. p., °C.	Recryst. Solvent	Nitrogen, % Calcd.	Found
246	CH_3	H	II	86	184–185	MeOH	6.09	6.12
358	C_6H_{11}[a]	H	IIA	75	150–151	Dil. i-PrOH	4.69	4.67
402	H	$CH_2CH{=}CH_2$	I	92	116–118	Dil. i-PrOH	5.47	5.46
33	H	CH_2CH_2OH	I	71	159–160	Water	5.38	5.38
133	H	$CH_2CH_2N(C_2H_5)_2$·HCl	IA	47	158–160	i-PrOH-ether	7.97[b]	7.77
206	H	$CH_2CH(OH)CH_3$	I	39	118–122	$CHCl_3$-heptane	5.11	5.14
248	CH_3	CH_2CH_2OH	I	60	94–95	Water	5.12	5.09
400	CH_3	$CH_2CH{=}CH_2$	I	85	93–94	Dil. i-PrOH	5.18	5.14
401	C_6H_{11}[a]	$CH_2CH{=}CH_2$	I	85	114–115	Dil. i-PrOH	4.14	4.17
372	C_6H_{11}[a]	$CH_2CH_2N(C_2H_5)_2$·HCl	IA	77	177–178	i-PrOH-ether	6.43[c]	6.47
135	$COCH_3$	$COCH_3$	..[d]	53	135–136	i-PrOH	4.67	4.65

[a] C_6H_{11} = Cyclohexyl
[b] Chloride analysis: Calcd., 10.08; Found, 10.20
[c] Chloride analysis: Calcd., 8.17; Found, 8.01.
[d] See Experimental Part.

salicylate (18), methyl 5-methoxysalicylate (19), 2,5-dimethoxybenzoic acid (20), and methyl 2-allyloxybenzoate (21).

5-Bromo-2-allyloxybenzamide (Method I).—A mixture of 32.4 Gm. (0.15 mole) of 5-bromosalicylamide, 18.2 Gm. (0.15 mole) of allyl bromide, 24 Gm. (0.175 mole) of anhydrous potassium carbonate and 200 cc. of acetone was stirred and refluxed for eight hours, and poured into 700 cc. of water. The mixture was made alkaline with sodium hydroxide, stirred for about ten minutes and filtered. The crude solid was washed with water and recrystallized from dilute isopropyl alcohol. Yield, 35.5 Gm. (92%); m. p., 116–118°.

In those instances in which the products were oils, the oils were extracted from the alkaline aqueous quench with ether or chloroform. After the ether solution had been washed and dried, the ether was removed and the residue was distilled at about 1 mm. pressure. Any isomerization which occurred during the distillation was detected by a positive ferric chloride test for phenol, and the isomer was removed by repeated extraction of an ether solution of the distillate with cold, dilute sodium hydroxide. The ether solution was then dried, and the ether evaporated at a low temperature to leave as a residue the allyloxy compound which gave a negative ferric chloride test.

When ethylene chlorohydrin was used as the alkylating agent, the reaction mixture, consisting of equimolecular quantities of amide, ethylene chlorohydrin and sodium hydroxide in water (150 cc. per 0.1 mole) and contained in a closed pressure bottle, was heated on a steam bath for about six hours. Solid products which separated from the cooled mixture were isolated and purified by recrystallization from an appropriate solvent. Oily products were extracted with immiscible solvent, such as chloroform, and purified by distillation.

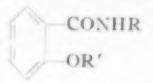
5-Bromo-2-(2-diethylaminoethoxy)benzamide Hydrochloride (Method IA).—5-Bromosalicylamide 10.8 Gm. (0.05 mole) was added to a solution of sodium methylate prepared from 1.1 Gm. (0.05 Gm. atom) of sodium and 20 cc. of methanol. Approximately 50 cc. of toluene was added, and after the mixture was distilled to remove the methanol, it was cooled and treated with 2.6 Gm. (0.04 mole) of 2-diethylaminoethyl chloride. The mixture was then refluxed for two hours, cooled, diluted with ether, and filtered. The layers were separated and the organic layer was washed with water and extracted with dilute hydrochloric acid. Neutralization of the acidic aqueous extract precipitated the base as a brown solid which was recrystallized from dilute methanol. There was so obtained 8.3 Gm. of base, m. p. 103–105°, which was converted into its hydrochloride by acidifying an alcoholic solution of the base with ethereal hydrogen chloride. Dilution of the solution with ether precipitated the hydrochloride salt as fine white needles. Yield, 8.4 Gm. (47%); m. p., 158–160°.

N-Methyl-5-bromosalicylamide (Method II).—A mixture of 57.8 Gm. (0.25 mole) of methyl 5-bromosalicylate, 62 Gm. (0.5 mole) of 25% aqueous methylamine and 60 cc. of methanol, contained in a pressure bottle, was heated at 90–100° for eight hours. The excess methylamine and most of the alcohol were removed by distillation, and the residual solution was poured into dilute hydrochloric acid. The solid which separated was collected on a filter, washed with water, and dried. Yield, 49.5 Gm. (86%); m. p., 184–185° after recrystallization from methanol.

Amidifications with ammonia were run in methanol and the mixtures were allowed to remain at room temperature for a period of about six days, after which they were worked up as described above.

5-Bromo-N-cyclohexylsalicylamide (Method IIA).—Twenty-nine and three-tenths grams (0.1 mole) of phenyl 5-bromosalicylate and 12.9 Gm. (0.13 mole) of cyclohexylamine were heated at 190–200° for three hours, and the liquid mixture was then distilled at 25 mm. to remove the phenol which had formed during the reaction. The brown, viscous residue was dissolved in hot isopropyl alcohol; the solution was treated with decolorizing charcoal, filtered, and diluted with water. The solid which separated from the cooled solution was recrystallized from dilute isopropyl alcohol to obtain 22.6 Gm. (75%) of small, white needles, m. p. 150–151°.

TABLE III.—SUBSTITUTED SALICYLAMIDES



Code No. S.2-	R	R	X	Method of Prepn.	Yield, %	M. p., °C.	Recryst. Solvent	Nitrogen, % Calcd.	Found
150	H	H	3,5-Diiso-propyl	II	72	116–117	Dil. i-PrOH	6.33	6.34
143	CH_2CH_2OH	H	3,5-Diiso-propyl	II	74	138–139	Dil. i-PrOH	5.28	5.18
529	C_4H_9	H	3,5-Diiso-propyl	II	59	50–51	Dil. MeOH	5.05	5.07
250	CH_3	H	3,5-Diiodo	II	82	215–216 (De-compn.)	i-PrOH	3.47	3.49
212	H	H	5-Hydroxy	II	30	217–218[a]	EtOH-CCl_4	9.15	9.23
230	CH_3	H	5-Hydroxy	II	64	208–209	Water	8.38	8.45
418	H	H	5-Methoxy	II	90	152–153	i-PrOH	8.38	8.44
503	H	$CH_2CH{=}CH_2$	5-Methoxy	I	72	78–79	Dil. i-PrOH	6.76	6.70
245	CH_3	H	5-Methoxy	II	80	114–115	Dil. EtOH	7.73	7.66
398	CH_3	CH_3	5-Methoxy	II	81	Oil[b]	.....	7.17	7.23
500	H	$CH_2CH{=}CH_2$	5-Allyloxy	I	70	85–86	Dil. EtOH	6.00	6.03

[a] After these studies were completed, the melting point of gentisamide was reported in the literature as 218–218.5° (9).
[b] B. p., 142–143° (1 mm.).

2-(β-Carboxyethoxy)benzamide (S.2-138).—To a solution of 13.7 Gm. (0.1 mole) of salicylamide and 4.0 Gm. (0.1 mole) of sodium hydroxide in 30 cc. of water at room temperature was added 7.2 Gm. (0.1 mole) of β-propiolactone over a period of twenty minutes. After remaining overnight, the reaction mixture was refluxed for one hour and acidified with dilute hydrochloric acid. The cooled mixture was filtered, and the solid was stirred with dilute sodium bicarbonate solution for about fifteen minutes. The unreacted salicylamide was removed by filtration, and the crude product was precipitated from the filtrate by acidification. Recrystallization of the crude material from water yielded 5.1 Gm. (24%) of white needles, m. p. 174–176°.

N-(4-Carboxyphenyl)acetylsalicylamide (S.2-220).—To a solution of 13.7 Gm. (0.1 mole) of *p*-aminobenzoic acid in 50 cc. of dioxane was added 10 Gm. (0.05 mole) of acetylsalicyloyl chloride (22) in 25 cc. of dioxane. The mixture was then heated at a temperature just below the boiling point for one hour, and poured into 500 cc. of water. The precipitate was collected on a filter and washed successively with 10% hydrochloric acid and water. The solid was taken up in hot acetone; the solution was treated with decolorizing charcoal, filtered, and diluted with water. A white solid separated on cooling. Yield, 5.5 Gm. (36%); m. p., 222–223° (decompn.).

N-Cyclohexyl-2-(2,3-dibromopropoxy)benzamide (S.2-326).—A solution of 3.2 Gm. (0.02 mole) of bromine in 25 cc. of carbon tetrachloride was combined with a solution of 5.2 Gm. (0.02 mole) of N-cyclohexyl-2-allyloxybenzamide (S.2-316). After remaining overnight at room temperature, the solution was washed with dilute sodium bisulfite solution and evaporated to an oil which solidified. The solid was recrystallized from dilute isopropyl alcohol to obtain 6.3 Gm. (75%) of material, m. p. 95–96°.

2-Diethylaminoethyl 2-(2-Cyclohexylcarboxamidophenoxy)acetate Hydrochloride (S.2-366).—A solution of 8.3 Gm. (0.03 mole) of 2-carboxymethoxy-N-cyclohexylbenzamide (S.2-364) and 4.1 Gm. (0.03 mole) of 2-diethylaminoethyl chloride in 50 cc. of isopropyl alcohol was refluxed for four hours and diluted with an equal volume of dry ether. The solid which separated on cooling was recrystallized from an isopropyl alcohol-ether mixture. Yield, 7.7 Gm. (62%); m. p., 171–172°.

O,N-Diacetyl 5-bromosalicylamide (S.2-135).—After a mixture of 21.6 Gm. (0.1 mole) of 5-bromosalicylamide, 30 Gm. (0.3 mole) of acetic anhydride and 25 cc. of pyridine had been refluxed for two hours it was poured onto a mixture of hydrochloric acid and ice. The precipitated solid was filtered off and recrystallized from 200 cc. of isopropyl alcohol. Yield, 16 Gm. (53%); m. p., 135–136°.

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Formation of Molecular Complexes by Some Water-Soluble Amides I.

Interaction of Several Amides With *p*-Hydroxybenzoic Acid, Salicylic Acid, Chloramphenicol, and Phenol*

By H. B. KOSTENBAUDER† and T. HIGUCHI

On the basis of previously proposed theories, molecules possessing amide groupings would be expected to exhibit ability to form molecular complexes similar to caffeine complexes with certain proton donors. Data are presented to show that amides formed between dimethylamine and several dibasic acids have this property. Both soluble and insoluble complexes seem to result when these agents are added to hydroxybenzoic acids, chloramphenicol, and phenol.

THE FORMATION of molecular complexes in aqueous solution by the interaction of pharmaceuticals with agents such as caffeine, polyethylene glycols, and polyvinylpyrrolidone has been considered in previous communications from these laboratories (1–8). The present study is directed toward the synthesis of new agents which would be expected to possess molecular complexing tendencies on the basis of previously proposed theories.

The carbonyl group of an amide, for example, is a good electron donor, and would be expected to show attraction for the electron-accepting groups of alcohols, phenols, amines, xanthines, sulfonamides, barbiturates, or other compounds having acidic hydrogen. These tendencies, as suggested earlier, are conducive to the formation of molecular complexes.

Since the presence of acidic hydrogen on unsubstituted amides results in association between the amide molecules themselves, the amides selected for this study were derivatives of dimethylamine. The compounds studied were N,N,N′,N′-tetramethylphthalamide, N,N,N′,N′-tetramethylisophathlamide, N,N,N′,N′-tetramethylterephthalamide, N,N,N′,N′-tetramethylfumaramide, and N,N,N′,N′-tetramethylsuccinamide. These compounds are white crystalline solids which are freely soluble in water and do not appear to exist in other than monomeric species in their aqueous solutions.

Observations were made on the complexing tendencies of these amides with *p*-hydroxybenzoic acid, salicylic acid, chloramphenicol, and phenol, utilizing the solubility and partition methods described in the caffeine studies (1, 4).

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† Fellow of the American Foundation for Pharmaceutical Education.

EXPERIMENTAL

Monomeric Nature of Species in Aqueous Solutions of the Amides.—The partition isotherms for the several amides, whose complexing tendencies were investigated, are illustrated in Fig. 1 and 2.

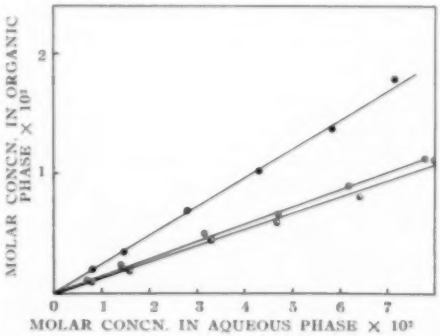

Fig. 1—Partition isotherms for: ●—N,N,N′,N′-tetramethylphthalamide; ⊗—N,N,N′,N′-tetramethylisophthalamide; ◐—N,N,N′,N′-tetramethylterephthalamide.

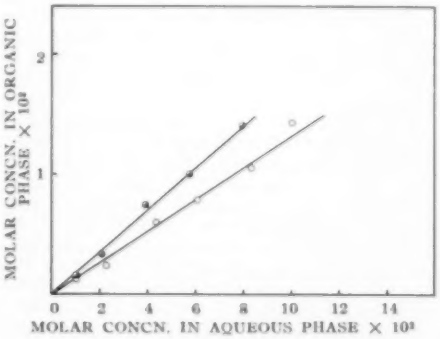

Fig. 2.—Partition isotherms for: ◑—N,N,N′,N′-tetramethylsuccinamide; O—N,N,N′,N′-tetramethylfumaramide.

The linearity of these partition isotherms indicates that the species in the aqueous phase are for all practical purposes the same as those in the organic phase, and in all probability are monomeric.

Interactions with p-Hydroxybenzoic Acid.—The complexing ability of the acid with the amides was studied using the solubility procedure described in earlier publications (2–5). The solubility of *p*-hydroxybenzoic acid in water at 30° in the presence of varying concentrations of N,N,N′,N′-tetramethylphthalamide, N,N,N′,N′-tetramethylisophthalamide, N,N,N′,N′-tetramethylterephthalamide, N,N,N′,N′-tetramethylfumaramide, and N,N,N′,N′-tetramethylsuccinamide as obtained by this method is illustrated in Fig. 3 and 4. All of these amides

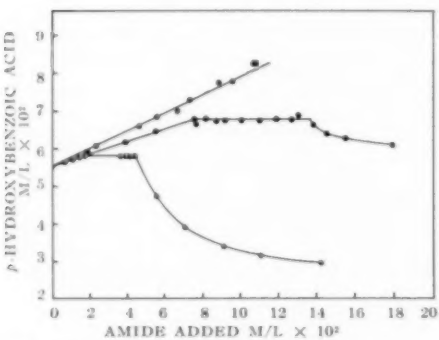

Fig. 3.—Solubility of *p*-hydroxybenzoic acid in water at 30° in the presence of: ⊗—N,N,N′,N′-tetramethylisophthalamide; ●—N,N,N′,N′-tetramethylphthalamide; ◐—N,N,N′,N′-tetramethylterephthalamide.

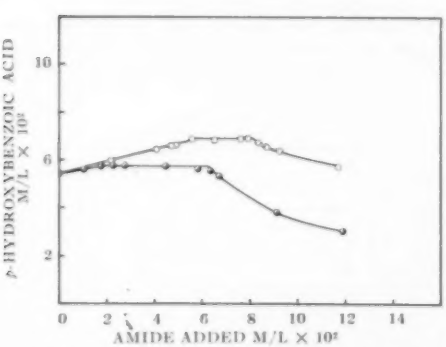

Fig. 4.—Solubility of *p*-hydroxybenzoic acid in water at 30° in the presence of: ○—N,N,N′,N′-tetramethylfumaramide; ◑—N,N,N′,N′-tetramethylsuccinamide.

exhibited definite complexing tendencies with *p*-hydroxybenzoic acid, the chief difference in the phase diagrams being due to the differences in the solubility of the complexes formed.

All of the compounds considered here appeared to form molecular complexes of the type

$$\text{Amide} + n\ \text{Acid} \rightleftharpoons \text{Amide} - (\text{Acid})_n.$$

The initial linear increase in the apparent solubility of *p*-hydroxybenzoic acid on the addition of the amide indicates that the principal complex being formed has a first order dependency with respect to the amide concentration. The number of acid molecules associated with each amide molecule in the complex cannot be determined by this method since the free acid concentration is an invariant. A true stability constant cannot be calculated without proof of the stoichiometry of the interaction, but an apparent stability constant can be calculated if the soluble complex is assumed to be effectively of a 1:1 ratio. The apparent stability constants calculated on this basis facilitate a comparison of complexing tendencies and are noted in Table I.

The more insoluble complexes exhibited plateau regions in the phase diagram. The plateau region results when the solution becomes saturated with respect to the complex. The total concentration of *p*-hydroxybenzoic acid in solution remains constant in this region, since the acid which is precipitated in the form of the complex is replaced by dissolution of the solid free acid which is present in excess. The length of the plateau region is dependent upon the amount of excess solid acid present in the system, and the ratio of the quantity of excess solid acid to the amount of amide added over the plateau region should correspond to the molecular ratio of acid to amide in the complex being precipitated.

Analysis of the phase diagram for the N,N,N′,N′-tetramethylphthalamide–*p*-hydroxybenzoic acid system gave a ratio of 2.4 *p*-hydroxybenzoic acid molecules to one amide molecule. Isolation and subsequent chemical analysis of the material being precipitated along the downcurve gave a ratio of 4.0 acid molecules to one amide molecule. The experimental downcurve does not correspond to the theoretical downcurve calculated on the assumption that the only complex occurring in solution is that which is precipitated over the plateau region. This information leads to the conclusion that there may be several simultaneous equilibria involving complexes of undetermined composition. The complex precipitated over the plateau region is not necessarily the same complex which is primarily responsible for the initial increase in solubility, but is simply the first complex to be formed in an amount in excess of its solubility.

The phase diagram for N,N,N′,N′-tetramethylterephthalamide and *p*-hydroxybenzoic acid gave a ratio of 3.0 molecules of *p*-hydroxybenzoic acid to one molecule of the amide. Chemical analysis of the material being precipitated along the downcurve showed variations for different samples, but gave a ratio of approximately 2.2 molecules of the acid to one molecule of the amide.

Complex formation between *p*-hydroxybenzoic acid and N,N,N′,N′-tetramethylisophthalamide did not result in a plateau region for the concentrations employed in this study. The apparent stability constant calculated on the basis of a 1:1 complex is included in Table I.

The system N,N,N′,N′-tetramethylfumaramide–*p*-hydroxybenzoic acid exhibited a plateau region which would indicate a complex of approximately 3.5

TABLE I.—STABILITY CONSTANTS FOR 1:1 COMPLEXES IN AQUEOUS SOLUTION AT 30°

	p-Hydroxybenzoic Acid	Salicylic Acid	Chloramphenicol	Phenol
N,N,N′,N′-tetramethylphthalamide	4.0[a]	2.0[a]	1.6[a]	...
N,N,N′,N′-tetramethylisophthalamide	5.7[a]	3.5[a]	2.7[a]	...
N,N,N′,N′-tetramethylterephthalamide	3.7[a]	2.6[a]	2.4[a]	...
N,N,N′,N′-tetramethylfumaramide	5.9[a]	4.7[a]	...	...
N,N,N′,N′-tetramethylsuccinamide	4.5[a]	2.2[a]	...	1.0

[a] These values should be considered as apparent stability constants since the studies on *p*-hydroxybenzoic acid and salicylic acid indicate that there may be simultaneous formation of complexes of molecular ratio other than 1:1.

acid molecules to one amide molecule. The ratio obtained upon chemical analysis of material precipitated along the downcurve was 2.7 acid molecules to one amide molecule.

The phase diagram for the interaction of N,N,N′,N′-tetramethylsuccinamide with *p*-hydroxybenzoic acid gave a ratio of 2.0 acid molecules to one amide molecule. Chemical analysis of the precipitate gave a ratio of 1.9 acid molecules to each amide molecule.

Interactions with Salicylic Acid.—A phase diagram showing the influence of N,N,N′,N′-tetramethylterephthalamide on the solubility of salicylic acid in water at 30° is presented in Fig. 5. The

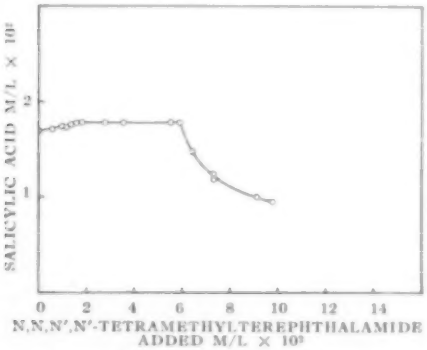

Fig. 5.—Solubility of salicylic acid in water at 30° in the presence of N,N,N′,N′-tetramethylterephthalamide.

increase in the solubility of salicylic acid in the presence of N,N,N′,N′-tetramethylphthalamide, N,N,N′,N′-tetramethylisophthalamide, N,N,N′,N′-tetramethylfumaramide, and N,N,N′,N′-tetramethylsuccinamide is illustrated in Fig. 6.

For the concentration conditions employed in this study, only the salicylic acid–N,N,N′,N′-tetramethylterephthalamide system exhibited a plateau region. Analysis of the phase diagram and chemical analysis of the insoluble material obtained along the downcurve indicated a complex composed of 3.0 acid molecules and one amide molecule. As in the *p*-hydroxybenzoic acid study, the complex primarily responsible for the initial increase in the solubility of salicylic acid is not necessarily the same complex which is being precipitated along the plateau. The principal complex responsible for the increased solubility in the presence of the amides can be considered to be effectively of molecular ratio 1:1, and apparent stability constants were calculated on this basis.

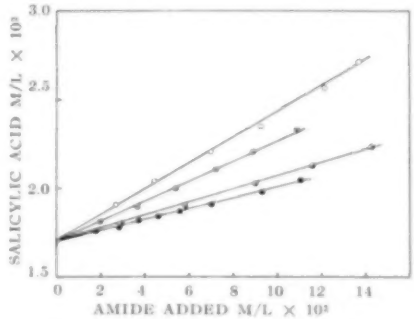

Fig. 6.—Solubility of salicylic acid in water at 30° in the presence of: ○—N,N,N′,N′-tetramethylfumaramide; ⊗—N,N,N′,N′-tetramethylisophthalamide; ◑—N,N,N′,N′-tetramethylsuccinamide; ●—N,N,N′,N′-tetramethylphthalamide.

Interactions with Chloramphenicol.—The solubility of chloramphenicol in water at 30° was studied as a function of the concentration of N,N,N′,N′-tetramethylphthalamide, N,N,N′,N′-tetramethylisophthalamide, and N,N,N′,N′-tetramethylterephthalamide. These data are presented in Fig. 7, and the apparent stability constants calculated on a 1:1 basis are included in Table I.

Interaction of Phenol and N,N,N′,N′-tetramethylsuccinamide.—The interaction of phenol and N,N,N′,N′-tetramethylsuccinamide was studied by

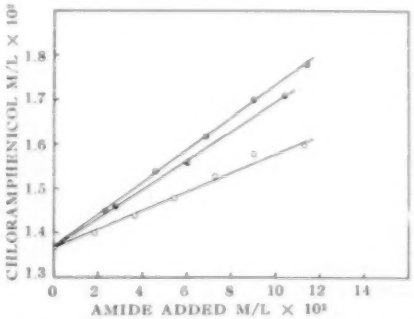

Fig. 7.—Solubility of chloramphenicol in water at 30° in the presence of: ⊗—N,N,N′,N′-tetramethylisophthalamide; ◐—N,N,N′,N′-tetramethylterephthalamide; ●—N,N,N′,N′ - tetramethylphthalamide.

a partition method similar to that used by Higuchi and Zuck (1) in the study of the caffeine-benzoic acid system. The other amides of this series could not be readily studied by a partition method since they were sufficiently soluble in the organic phase to invalidate the assay for phenol.

The partition system employed in this study was water-iso-octane (2,2,4-trimethylpentane), phenol being present in both phases while the amide remained in the aqueous phase.

The phenol concentration in the iso-octane layer was determined spectrophotometrically, and by means of the partition coefficient for phenol in this particular system, it was possible to calculate the concentration of free phenol in the aqueous phase. By assuming that the reaction of phenol and the amide proceeded on a 1:1 basis, it was then possible to obtain the respective concentrations of complex and free amide. The stability constants calculated for the reaction:

$$\text{Phenol} + \text{Amide} \rightleftharpoons \text{Phenol-Amide}$$

were found to be apparently constant over the concentration range studied. The concentration of complexed phenol as a function of added N,N,N′,N′-tetramethylsuccinamide is shown in Fig. 8.

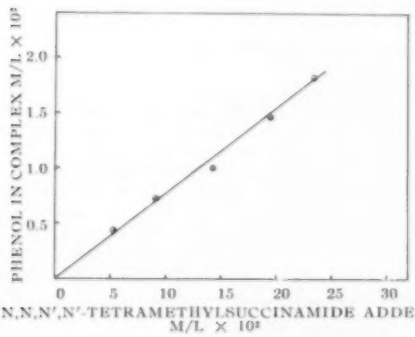

Fig. 8.—Interaction of phenol with N,N,N′,N′-tetramethylsuccinamide in water at 30°.

GENERAL DISCUSSION

Evidence was obtained for the formation of definite complexes between several amides and compounds with which these agents would theoretically be expected to interact. In all cases the concentration of the principal soluble species formed from the interaction of the amides with *p*-hydroxybenzoic acid, salicylic acid, chloramphenicol, and phenol appeared to have a first order dependency with respect to amide concentration. In the interaction of *p*-hydroxybenzoic acid with N,N,N′,N′-tetramethylphthalamide, N,N,N′,N′-tetramethylterephthalamide, N,N,N′,N′-tetramethylfumaramide, and N,N,N′,N′-tetramethylsuccinamide, and the interaction of salicylic acid with N,N,N′,N′-tetramethylterephthalamide there was evidence for the formation of relatively insoluble complexes containing several acid molecules for each amide molecule.

The complexes formed between the amides and salicylic and *p*-hydroxybenzoic acids are qualitatively similar to those observed in the interaction of caffeine with these acids (6). The solubilities of the complexes formed by members of the tetramethylphthalamide series, however, do not necessarily parallel the solubilities of the complexing agents themselves. The relative solubilities are N,N,N′,N′-tetramethylphthalamide > N,N,N′,N′-tetramethylisophthalamide > N,N,N′,N′-tetra-methylterephthalamide.

The stability constants for the complexes formed by the amides cannot be directly compared with the constants for complexes formed by the xanthines since the constants for the amides are apparent values calculated on the basis of 1:1 interaction, while there is evidence which indicates that there are other interactions occurring simultaneously.

The results of these studies on the amides suggest that the formation of molecular complexes between many types of organic compounds in aqueous solution may be a relatively common although generally unsuspected occurrence.

PROCEDURE

Preparation of Amides.—The amides were prepared by treating an ether solution of succinyl chloride, and benzene solutions of the other acid chlorides, with dimethylamine. The amide was then extracted with water and the aqueous layer was treated with sodium carbonate to neutralize any acid resulting from unreacted acid chloride. The aqueous layer was extracted with chloroform, and the amide was recovered on evaporation of the chloroform. The amide was then purified by recrystallization.

Reagents.— N,N,N′,N′-tetramethylphthalamide, m. p. 122–123°;[1] N,N,N′,N′-tetramethylisophthalamide, m. p. 137–138°; N,N,N′,N′-tetramethylterephthalamide, m. p. 200–201°; N,N,N′,N′-tetramethylfumaramide, m. p. 130–131°; N,N,N′,N′-tetramethylsuccinamide, m. p. 83–86°; *p*-hydroxybenzoic acid, m. p. 216°; salicylic acid, m. p. 162°; phenol, b. p. 182°; chloramphenicol, m. p. 151–152°; 0.1 *N* alcoholic potassium hydroxide solution; bromthymol blue and methyl red as indicators.

***p*-Hydroxybenzoic Acid.**—Excess quantities of the acid were placed in 125-ml. glass-stoppered bottles together with varying quantities of the amide being studied and 50 ml. of 0.005 *N* sulfuric acid solution. The dilute acid solution was employed to suppress any ionization of the *p*-hydroxybenzoic acid. The bottles were agitated in a constant temperature bath at 30° for fourteen hours. Aliquot portions of the supernatant liquid were then removed and analyzed for acid content by titration with 0.1 *N* alcoholic potassium hydroxide solution, with bromthymol blue as the indicator. The quantities of acid used were as follows: N,N,-N′N′-tetramethylphthalamide, 1.5 Gm. acid; N,N,N′,N′-tetramethylisophthalamide, 1.0 Gm. acid; N,N,N′,N′-tetramethylterephthalamide, 1.0 Gm. acid; N,N,N′,N′-tetramethylfumaramide, 1.0 Gm. acid; N,N,N′,N′-tetramethylsuccinamide, 1.0 Gm. acid.

Salicylic Acid.—The procedure was identical to that described for *p*-hydroxybenzoic acid with the exception that methyl red was used as the indicator. The quantities of salicylic acid used were as follows:

[1] All melting points uncorrected.

N,N,N′,N′-tetramethylphthalamide, 0.5 Gm. acid; N,N,N′,N′-tetramethylisophthalamide, 1.0 Gm. acid; N,N,N′,N′-tetramethylterephthalamide, 1.0 Gm. acid; N,N,N′,N′-tetramethylfumaramide, 0.5 Gm. acid; N,N,N′,N′-tetramethylsuccinamide, 0.5 Gm. acid.

Chloramphenicol.—An excess quantity of chloramphenicol (0.5 Gm.) was placed in a 125-ml. glass-stoppered bottle with varying quantities of the amide to be studied and 50 ml. of distilled water. The bottles were then agitated in a constant temperature bath at 30° for fourteen hours. Samples were then removed and the quantity of chloramphenicol in solution was determined spectrophotometrically at a wavelength of 300 mμ for the N,N,N′,N′-tetramethylphthalamide study, 305 mμ for the N,N,N′,N′-tetramethylisophthalamide study, and 310 mμ for the N,N,N′,N′-tetramethylterephthalamide study.

Phenol.—A partition system composed of 0.5 Gm. of phenol, 25 ml. of distilled water, 25 ml. of iso-octane, and varying quantities of N,N,N′,N′-tetramethylsuccinamide in 125-ml. glass-stoppered bottles was employed. The bottles were agitated in a constant temperature bath at 30° for several hours. Samples were then removed from the iso-octane layer and the phenol concentration in this layer was determined spectrophotometrically at 265 mμ.

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Studies on Gentiopicrin*

By SIRKKU PESONEN† and EGIL RAMSTAD

Pure gentiopicrin, when exposed to oxygen, is oxidized spontaneously to an acidic substance "gentiamarin." In the presence of moisture this acidic contaminant speeds up the hydrolytic decomposition of gentiopicrin as well as of itself. Infrared-spectroscopic and paper-chromatographic studies were made of gentiopicrin, gentiamarin, and their hydrogenation products. A structural formula is proposed for gentiamarin.

GENTIOPICRIN, the bitter principle of gentian root, has repeatedly been the object of chemical investigations. Tanret (1) established, in 1906, the glycosidic nature of the compound and put forth the empirical formula of $C_{16}H_{20}O_9$. He also found that gentiopicrin is a lactone capable of forming salts with alkali. These salts, in contrast to the lactone, are not bitter. By hydrolysis with emulsin the glycoside gave a precipitate, the gentiogenin, and the sugar glucose:

$$C_{16}H_{20}O_9 + H_2O \longrightarrow C_{10}H_{10}O_4 + C_6H_{12}O_6$$

Asahina, *et al.* (2, 3), concluded from their investigations that the aglycone of gentiopicrin exists in three different forms, one of which, the mesogentiogenin, is the primary hydrolytic product and is subsequently isomerized, partly into eugentiogenin and partly into Tanret's gentiogenin. The latter is probably a dimer. A study of various derivates of gentiopicrin indicated that the aglycone, as it exists in the glycoside, is still another distinct compound, which they termed protogentiogenin. Asahina is of the opinion that protogentiogenin is stable only as it exists in combination with glucose.

In this paper a report is made of chromatographic studies of the products of gentiopicrin resulting from enzymatic hydrolysis, and from exposure of gentiopicrin to the air.

EXPERIMENTAL

Material.—The gentiopicrin was extracted from stabilized gentian root[1] and made available by Alpert and Ramstad (4). M. p. 176–177° (Kofler); $[\alpha]_D^{25} = -200.0°$. Repeated recrystallization from alcohol and from water did not change the m. p. or the optical activity, nor did purification by paper chromatography.

Testing of Instability of Gentiopicrin.—Gentiopicrin was chromatographed on Whatman No. 1 paper with the organic phase of a mixture of butanol, acetic acid, and water (40 + 10 + 50) by descending technique. The developing spray was aniline hydrogen phthalate (5). Result: besides gentiopicrin ($R_f = 0.58$) a small spot of glucose resulted ($R_f = 0.12$), and likewise a small spot at $R_f = 0.19$

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Presented to the Scientific Section, A. PH. A., Miami Beach meeting, May 1955.
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[1] Kindly furnished by Professor H. Flück, Eidgenossische technische Hochschule, Zurich, Switzerland.

The latter is designated in the following work as "gentiamarin."

The gentiopicrin was eluted carefully from the cut-out spot at room temperature, the solution reapplied to a new chromatographic sheet and rechromatographed in the same way as above. After development of the chromatogram with the aniline hydrogen phthalate spray, the same three spots (R_f = 0.12, 0.19, and 0.58) again appeared.

Two hundred mg. of gentiopicrin was dissolved in alcohol, the entire solution was applied as a 5-mm. wide streak across Whatman No. 1 paper, dried, and then the sheets were chromatographed. Narrow strips were cut longitudinally from the chromatograms and developed with the aniline hydrogen phthalate spray to locate the gentiopicrin. The gentiopicrin streak was cut out, eluted with alcohol and concentrated in vacuum without use of heat. The resulting gentiopicrin had a m. p. of 176-177°.

Chromatograms of gentiopicrin were allowed to stand in closed containers for two to seven days. By spraying the chromatograms with an alcoholic, 0.1% solution of bromcresol green, acid reaction was now exhibited in the area of the chromatogram occupied by gentiopicrin. A recently prepared chromatogram displayed no acidity of the gentiopicrin area. Heating of the gentiopicrin spot in the air for half an hour likewise led to its conversion to an acidic substance.

Gentiopicrin spots were exposed to air for one week, eluted with alcohol, and rechromatographed with the same solvent system as above. Besides gentiopicrin (R_f = 0.58) and a small amount of glucose (R_f = 0.12), an acid spot appeared at R_f = 0.19.

Chromatography of the Several Forms of Gentiopicrin Aglycone.—One gram of gentiopicrin was dissolved in 20 ml. of water. Thirty mg. of highly purified emulsin and a drop of toluene were added, and the mixture allowed to stand for three days at 28°.

The chromatographic solvent system consisted of a mixture of chloroform-benzene (4 + 6), saturated with formamide (7, 8). Whatman No. 1 paper was impregnated with a mixture of formamide and acetone (2 + 8) and air-dried immediately before spotting. After development, the sheets were air-dried and inspected under ultraviolet light without spray treatment. See Table I.

TABLE I.—PAPER CHROMATOGRAPHY OF THE HYDROLYZING MIXTURE OF GENTIOPICRIN. DESCRIPTION OF ZONES

Zone Designation	R_f Value	Color: In White Light	Color: In Ultraviolet Light
A_1 (Starting line)	0.00	Brownish	White
A	0.03	....	Greenish
B	0.09	....	Pink
C	0.12	....	Light lilac
D	0.20	....	Bluish
E (Streaking)	..	....	Light blue
F	0.69	Reddish brown[a]	Light yellow

[a] Upon standing in air, spot F gradually turned reddish brown in the course of a few days.

Attempted Isolation of the Aglycone Forms.—Spots A_1, A, B, D, E, F were eluted separately with 95% alcohol in a nitrogen atmosphere, and the solutions concentrated in vacuum without use of heat. No crystals were obtained from any of the solutions. The individual fractions were then rechromatographed alongside a hydrolyzing mixture of gentiopicrin. The above benzene-chloroform-formamide solvent system was used. The R_f values of the rechromatographed substances were 0.00. When the butanol-acetic acid-water solvent system was used, substances A_1, A, B, and D were left on the origin, while substance F had been transformed to yield several different spots.

Hydrogenation.—Hydrogenation was carried out by use of the method and apparatus of Noller and Barusch (7). Catalyst: platinum oxide; hydrogenation time: seven to forty-eight hours; temperature 27°. Gentiopicrin 0.237 Gm.: 53.5 ml. H_2; gentiopicrin 0.178 Gm.: 31.3 ml. H_2.

Paper Chromatography of Hydrogenation Products of Gentiogenin.—Solvent system: butanol–acetic acid–water; developing spray; aniline hydrogen phthalate. The following spots appeared: R_f = 0.12 (glucose), 0.51 (τ), 0.66 (ϵ). Nonsprayed spot of ϵ turned pink upon prolonged exposure in the air. Spots τ and ϵ were treated with emulsin according to the method of Bliss and Ramstad (8). Chromatography in a second direction revealed that glucose had formed from both spots. Furthermore, spot ϵ also gave rise to an aglycone detectable with aniline hydrogen phthalate spray and heat. Heating alone caused the aglycone spot of ϵ to turn pink. Its R_f was 0.78.

DISCUSSION

It was noted early by Tanret (1) that gentiogenin is destroyed rather rapidly in gentian root. The preparation of pure gentiopicrin by many authors has offered difficulties (2, 3, 6). Korte (6) states that the extract of gentian root must be worked up in the course of 4 days or the isolation of the glycoside will be unsuccessful. However, some gentiogenin can be obtained even from a greatly deteriorated extract by shaking the glycoside out from aqueous solutions with chloroform containing 25-30% alcohol (4). Our experiments show that pure gentiopicrin is hydrolyzed to a considerable extent in the course of elution from the chromatograms with alcohol at room temperature so that glucose becomes a contaminant of gentiopicrin together with aglycones, and in addition, a new substance (gentiamarin) is formed. This compound is an acid and, possibly, is identical with Tanret's gentiamarin. It forms particularly readily when gentiopicrin is exposed in thin layers to the atmosphere. Oxygen is necesary for its formation, because it does not form when gentiopicrin is stored in a nitrogen atmosphere. Gentiamarin is itself a glucoside since it yields glucose upon hydrolysis. It was found that this secondary glycoside hydrolyzed rather fast autocatalytically. The presence of this acid in gentiopicrin preparations certainly is a contributing factor to the further deterioration of gentiopicrin since its acid properties would speed up the hydrolysis of gentiopicrin. For this reason it is questionable if gentiopicrin has ever been obtained in an absolutely

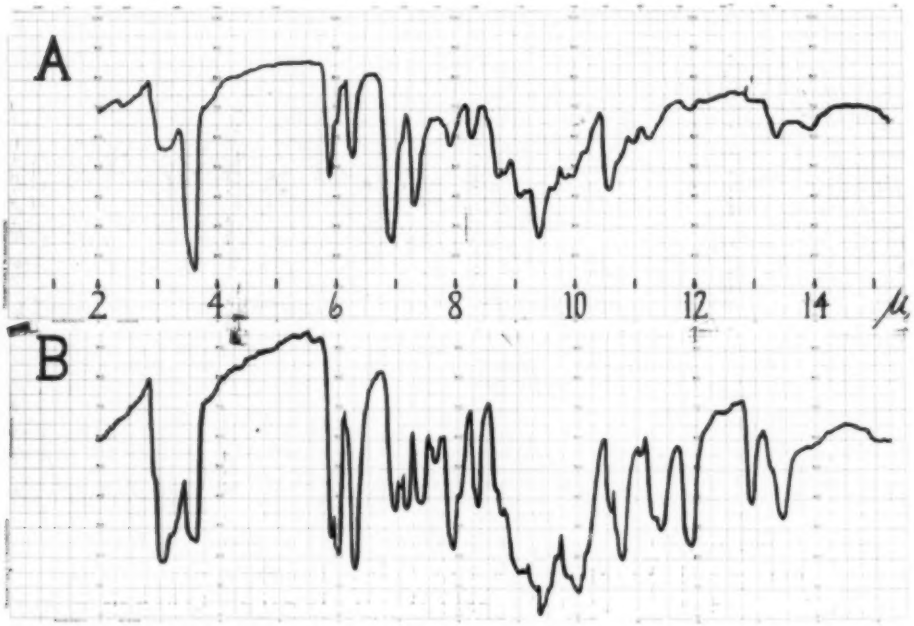
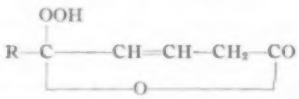


Fig. 1.—Infrared absorption spectra. A, gentiopicrin; B, gentiopicrin exposed to air in thin layers.

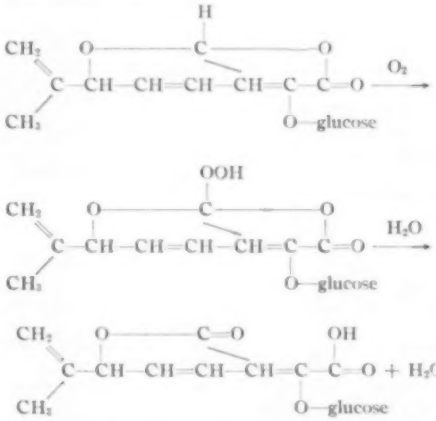
pure form. Infrared analysis of the purest sample of gentiopicrin which we could make, i. e. crystalline gentiopicrin purified by paper chromatography and stored under nitrogen in a deep-freeze, and of the same sample left in air for three weeks (exposed gentiopicrin) gave the following information on the chemical changes which take place during exposure to air[2] (Fig. 1).

In the exposed gentiopicrin, the strongly increased peak between the wavelengths of 2.84 μ to 3.22 μ (3.05 μ to 3.08 μ) would indicate a considerable increase of carboxyl groups in the product formed from gentiopicrin upon standing in air. In addition to gentiamarin the presence of small amounts of glucose in exposed gentiopicrin might explain the increased absorption of infrared radiation at the wavelengths. A considerable increase in the absorption at 6.00 μ is consistent with the formation of the carboxyl group which appears to take place during exposure of gentiopicrin to the air. A strong peak of 5.88 μ in the spectra of both samples is an indication of the carbonyl group. The strong absorption at 10.75 μ, 11.90 μ, and 12.92 μ corresponds to those produced by pure glucose and indicates the presence of free glucose in the exposed gentiopicrin.

Kirkland, *et al.* (9) have found that β, γ-angelica lactone readily forms peroxides,



also that α,β-lactone forms peroxide, although slower. The formation of peroxide in this process is followed by secondary reactions. Gentiopicrin is an α,β-lactone, and the behavior of this compound as shown above leads to the belief that an analogous oxidation of it furnishes gentiamarin.



Upon hydrogenation gentiopicrin consumes 6 hydrogen atoms. The hydrogenation products contain, in addition to some glucose resulting from hydrolysis, two glucosides, both of which are hydrolyzable with emulsin. Evidently, one of them is the hexahydrogentiopicrin, the other hexahydrogentiamarin. As expected, the latter is subject to a rather rapid hydrolysis as a result of its acid character, since it contains a free carboxyl group. The hexa-

[2] We are greatly indebted to Dr. W. F. Edgell, Department of Chemistry, Purdue University, for help in interpreting the infrared spectra.

hydrogentiopicrin furnishes an aglycone (hexahydroprotogentiogenin?) which gives an aldehyde reaction and, upon exposure to heat on a chromatogram, gives a pink color. Thus, the hydrogenation experiments give further support to the view that gentiamarin has the structure as proposed above.

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The Effect of *p*H upon the Solubility of Phenobarbital in Alcohol-Aqueous Solutions*

By ARNOLD URDANG† and E. EMERSON LEUALLEN‡

The solubility of phenobarbital in combinations of alcohol and water at various *p*H values has been determined and is herewith reported. It was found that acid *p*H values have little effect upon the solubility of phenobarbital as the alcohol concentration is increased; whereas, hydrogen ion concentrations of 10^{-7} to 10^{-10} do cause a marked increase. A table and figure are included for use in determining quickly the volume of alcohol required to dissolve a given weight of phenobarbital when the *p*H is known.

ONE OF THE MANY problems encountered in pharmacy is the determination of the quantity of alcohol necessary to maintain a required amount of phenobarbital in solution. Previous investigators have shown the effect hydroalcoholic (1), glycerol-alcohol-water (2), and propylene glycol-alcohol-water (3) systems have upon the solubility of phenobarbital. Higuchi, *et al.* (4), studied the relationship that *p*H has upon the solubility of slightly acidic drugs including phenobarbital. His values, pertaining to phenobarbital, are relatively low as compared to the work of the previous investigators which may be due to the fact that the percentage was calculated on a w/w basis whereas all of the other authors used a w/v relationship. Since pharmaceuticals vary so greatly in hydrogen ion concentration, it was felt that the relationship which *p*H plays with regard to this problem should be expanded to cover a wide range of *p*H values as well as various concentrations of alcohol.

Therefore, it was decided to determine the solubility of phenobarbital at various concentrations of alcohol from 0 to 50 per cent and at *p*H values from 2 to 10, and from this information prepare a table and graph to facilitate use of this data.

Since we were dealing with solutions of phenobarbital, some of which were basic in nature, the question of stability had to be considered. Nielson (5) determined the deterioration of a 10 per cent solution of sodium phenobarbital at a *p*H of 9.4 and reported a 1 per cent decomposition in two months at 1°, and a 1 per cent decomposition in three weeks at 20°. This would seem to indicate that the degradation of phenobarbital does not have to concern us since the solutions with which we worked were kept for a maximum of forty-eight hours during which time an insignificant amount of phenobarbital can be considered decomposed.

The usual method of determining the solubility of a compound by evaporation of the solvent was not feasible since the buffers present would crystallize upon evaporation of the solvent. A satisfactory spectrophotometric assay was developed adopting the procedure described in the A.O.A.C. (6). Walker, *et al.* (7), reported that

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the absorbance of ultraviolet light by phenobarbital at 241 mμ increases with a rise in *p*H. Therefore, it was necessary to prepare nine standard curves, one for each *p*H value from 2 to 10.

The hydrogen ion concentration was determined by means of a Beckman *p*H meter using a No. 015 glass electrode for *p*H values below 9, and a No. 1190E glass electrode for *p*H values 9 and 10. Izmailov and Belgova (8) reported that the potential of a glass electrode in alcohol-aqueous solutions with a low *p*H value does not differ from that of aqueous solution with the same activity of hydrogen ions. Deviations from linearity occur at *p*H 9.5 for 40 per cent alcohol and *p*H 8 for 50 per cent alcohol. However, it was felt that a correction would not be necessary since the error involved below a *p*H of 11 was not very large.

TABLE I.—SOLUBILITY OF PHENOBARBITAL IN ALCOHOL-AQUEOUS SYSTEMS AT VARIOUS *p*H VALUES[a]

% Alcohol (v/v)	*p*H 2-6	7	8	9	10
0	0.11[b]	0.17	0.41	1.40	2.90
5	0.12	0.18	0.41	1.19	2.71
10	0.16	0.22	0.41	1.01	2.53
15	0.20	0.26	0.41	0.94	2.34
20	0.27	0.33	0.49	0.94	2.15
25	0.40	0.47	0.63	1.01	1.99
30	0.61	0.70	0.85	1.15	1.98
35	0.94	1.03	1.16	1.40	2.07
40	1.46	1.45	1.60	1.80	2.28
45	1.94	2.10	2.20	2.36	2.64
50	2.68	2.84	3.02	3.20	3.49

[a] The solubility was determined at 25 ± 0.02°.
[b] Denotes % phenobarbital (w/v).

The Gifford buffer system (9) was chosen to prepare the proper *p*H solution since it did not interfere with the spectrophotometric assay. It was found that those systems employing the use of organic salts were not practical since they absorbed ultraviolet light and thereby complicated the assay procedure. The alcoholic buffers were prepared by taking the aqueous *p*H solutions and adding the necessary quantity of alcohol to achieve the desired per cent by volume solutions. These solutions were then checked for the proper *p*H and adjusted whenever necessary with 0.1 per cent HCl.

EXPERIMENTAL

Approximately 50 ml. of the appropriate buffer solution was transferred to a four-ounce bottle, and between 2.5 and 3.0 Gm. of phenobarbital U. S. P. was added. A rubber stopper was then inserted and the vials were placed in a constant temperature bath at 25° ± 0.02° and rotated for forty-eight hours. At the end of this period, two aliquots of the clear liquid were withdrawn, diluted with the corresponding buffer solution, and analyzed according to the procedure indicated. Each set of alcohol concentrations, from 0 to 50%, was run in a similar manner.

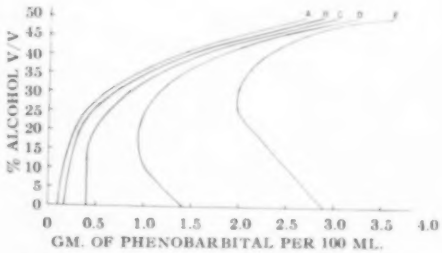

Fig. 1.—Solubility of phenobarbital in alcohol-aqueous solutions at *p*H values from 2 to 10. *A* = *p*H 2 to 6; *B* = *p*H 7; *C* = *p*H 8; *D* = *p*H 9; *E* = *p*H 10.

SUMMARY

1. A determination of the effect *p*H has upon the solubility of phenobarbital in alcohol-aqueous solutions at 25° has been presented.

2. A table and figure have been included to facilitate use of these data.

3. The solubility of phenobarbital in hydroalcoholic solutions at a *p*H of 9 and 10 reaches a minimum at 18 and 28% alcohol, respectively.

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A Study of the Effect of Nonionic Emulsifying Agents on the Release of Medication from Hydrophilic Ointment Bases*

By DONALD Y. BARKER, H. GEORGE DeKAY, and JOHN E. CHRISTIAN

Hydrophilic ointment U. S. P. XIV has been reported to possess undesirable compatibility and irritation properties due to the presence of the anionic surfactant, sodium lauryl sulfate in the formulation. This paper describes the effect of replacing sodium lauryl sulfate with nonionic surfactants in hydrophilic ointment on the release of medication from the ointment bases when tested by the agar plate method.

HYDROPHILIC OINTMENT U. S. P. XIV has been reported (1) to possess undesirable compatibility and irritation properties due to the presence of the anionic surfactant, sodium lauryl sulfate in its formulation. Accordingly, the Revision Committee of the U. S. P. recommended the study of this ointment base in an effort to overcome this disadvantage by the use of some available nonionic surfactants.

Barr, *et al.* (2), and Lesshafft and DeKay (3) have undertaken this project by screening the available nonionic surfactants for their emulsifying ability with the subsequent evaluation of the formulations for desirable physical and compatibility properties. It is considered equally as important to know the effect of the various surfactants, in varying concentrations, on medicament release; accordingly, this project was undertaken.

The literature offers a multiplicity of procedures for the determination of the release of medication from ointment bases. The official agar plate method (4), however, does hold a prominent position with regard to frequency of usage for such a purpose. Therefore, this established procedure was employed for this study.

EXPERIMENTAL

Choice of Surfactants.—From the preliminary experimentation by Lesshafft and DeKay (3) ten nonionic surfactants were noted to exhibit an enhanced ability to form stable emulsion-type ointment bases. Therefore, these nonionic surfactants: Polyethylene glycol 1000 monostearate, G-1045,[1] G-7596-P,[1] Brij 35,[1] Tween 60,[1] Tween 40,[1] Tween 20,[1] Myrj 49,[1] G-7596-J,[1] and Ethomid C/15[2] were selected in this study.

Preparation of the Ointment Bases.—Hydrophilic ointment was prepared as directed in the U. S. P. XIV (5) and the test ointment bases were prepared by substituting the sodium lauryl sulfate with the nonionic surfactants in four concentrations: 1, 3, 5 and 7%. In the formulations employing a 1% concentration of nonionic surfactant, no change was made in the formulation except for the surfactant substitution. When the concentration of the nonionic surfactant exceeded 1%, a reduction of equal parts of each was made in the stearyl alcohol and petrolatum, respectively. The water content in all formulations was unaltered. After the ointments had congealed, they were passed through the Kent Roller Mill[3] to improve their consistency before the incorporation of the medicinal agent.

Since the principle of the agar plate method depends upon the bacteriostatic effect of the incorporated medicament on a standard organism culture, it was necessary to select medicaments that would exert a definite bacteriostatic action. Accordingly, two medicaments, mercuric oxide and iodine, were selected for this study. The mercuric oxide was incorporated by levigation. The iodine was incorporated by first dissolving it in a potassium iodide solution. The potassium iodide solution was prepared by dissolving sufficient potassium iodide in one to two drops of water to make a final ointment concentration of 1%. The iodine-potassium iodide solution was incorporated by levigation.

Each ointment base was tested using the two medicinal agents in a 1% concentration, respectively.

Testing the Ointments by the Agar Plate Method.—Sterile nutrient broth, 50 ml., containing 0.5% of Bacto beef extract,[4] 0.5% of sodium chloride[5] and 1% Bacto peptone[4] was inoculated with the organism *Micrococcus pyogenes* var. *aureus* 209P.[6] This inoculated broth was incubated for twenty-four hours at 37° after which a 0.5-ml. transfer was made to another 50 ml. of sterile nutrient broth and incubated for twenty-four hours.

Ten ml. of sterile nutrient agar, prepared by dissolving 1.5% of Bacto agar[4] in nutrient broth was transferred to individual sterile petri dishes using an automatic pipetting Brewer machine.[7] Four sterile penicillin assay cups, having an external diameter of 8 mm. were spaced uniformly on the

* Received April 5, 1956, from the School of Pharmacy, Purdue University, Lafayette, Ind.
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[1] Trademark name of Atlas Powder Co., Wilmington, Delaware.

[2] Trademark name of Armour and Co., Chicago, Ill.

[3] Available from Kent Machine Works, Inc., Brooklyn, N. Y.

[4] Available from Difco Laboratories, Detroit, Mich.

[5] Sodium chloride, powdered, U. S. P.

[6] Obtained from Bacteriology Department, Purdue University, Lafayette, Ind.

[7] Available from Baltimore Biological Laboratories, Baltimore, Md.

solidified agar. To each petri dish was then added 15 ml. of the nutrient agar, inoculated with 0.1 ml. of the standard organism per 20 ml. of sterile nutrient agar. When the agar had solidified, the penicillin assay cups were removed by a slight twisting action leaving four uniform cups in the agar medium.

To expedite the placement of the ointment sample in the agar cups, the ointments were dispensed in one-dram collapsible tubes. Starting at the bottom of the agar cup, the ointment was applied slowly in circular movements until the cup was completely filled. By maintaining a positive pressure between the nozzle of the ointment tube and the ointment sample being dispensed, the cup was uniformly filled assuring an intimate contact between the ointment sample and the agar surface.

Reproducible, 200-mg. samples of ointment agreeing within ± 2 mg. were dispensed to each cup. Preliminary experimentation indicated that this variation did not produce significant differences in the resulting zones of inhibition.

During each daily plating procedure, the following plates were subjected to testing: two plates, each containing four samples of the medicated ointment base being tested, one plate containing the medicated hydrophilic ointment U. S. P. XIV as a standard, one plate containing the ointment base but no medicament to check the bacterial inhibitory effect of the surfactant, one plate containing no ointment to check for inhibition of the growth of the organism caused by the solutions employed in the preparation of the plates, and one plate containing no organism to check for contamination of the solutions or equipment.

The plates were inverted and placed in the incubating oven at 37° for a period of twenty-four hours. At the end of the incubation period, the plates were removed from the oven and diameters of the zones of bacterial inhibition determined.

The diameters of the zones of inhibition for the eight samples of each ointment base tested were determined by placing the inverted plate on a Darkfield Quebec Colony Counter[5] and measuring the two perpendicular diameters of each zone using a millimeter rule under a high powered lens. The two diameters for each sample of ointment were averaged, and the mean diameter for the eight samples of each ointment base were calculated. This plating procedure was repeated on separate days until 10 readings of the mean diameter for the eight samples of each ointment base were reproduced to within ±1 mm. of the mean of the 10 readings. This represented the results of a minimum of 80 samples for each ointment base tested. The mean zone of inhibition from the results of the 10 readings, corrected for the area of the ointment sample are indicated in Table I.

TABLE I.—THE EFFECT OF DIFFERENT SURFACTANTS STUDIED IN VARYING CONCENTRATION ON THE ZONES OF BACTERIAL INHIBITION PRODUCED BY MERCURIC OXIDE AND IODINE IN HYDROPHILIC OINTMENT U. S. P. XIV

Surfactant[a]	Medicament[b]	Area of Zone of Inhibition,[c] sq. mm. Concentration of Surfactant, % 1	3	5	7	Sum of Areas, sq. mm.
PEG. 1000 monostearate	Mercuric oxide	460.6	452.6	365.3	429.1	1,946
	Iodine	296.2	239.4	80.5	117.2	733
Tween 40	Mercuric oxide	501.5	421.3	448.7	440.8	1,885
	Iodine	402.3	210.0	210.0	153.4	975
Myrj 49	Mercuric oxide	460.7	409.8	361.7	417.5	1,870
	Iodine	372.6	239.4	82.5	114.9	809
G-7596-J	Mercuric oxide	476.8	460.6	489.0	468.7	1,837
	Iodine	306.2	221.5	358.1	136.0	1,021
Ethomid C/15	Mercuric oxide	476.8	460.6	464.6	468.7	1,812
	Iodine	372.6	171.5	236.3	105.9	886
Brij 35	Mercuric oxide	518.3	480.8	472.7	476.8	1,777
	Iodine	264.0	174.1	153.4	168.8	760
G-7596-P	Mercuric oxide	448.7	387.3	484.9	456.6	1,740
	Iodine	276.7	163.6	143.4	174.1	758
Tween 60	Mercuric oxide	440.8	417.5	379.8	448.7	1,707
	Iodine	276.7	190.3	140.9	193.1	801
Tween 20	Mercuric oxide	472.7	421.3	413.7	432.9	1,686
	Iodine	283.1	176.8	163.6	145.8	769
G-1045	Mercuric oxide	440.8	460.6	447.7	488.7	1,649
	Iodine	267.1	316.3	150.8	119.5	853
Sodium lauryl sulfate[d]	Mercuric oxide	316.3	...	...	...	...
	Iodine	227.4	...	...	...	...

[a] The surfactants possessed no bacteriostatic activity indicated by the absence of zones of inhibition when the ointment bases containing no medicinal agent were tested for their ability to inhibit bacterial growth. During the testing procedure there was no evidence of inhibition of bacterial growth due to the solutions employed in the test procedure, as indicated by the normal growth of the organism when the inoculated nutrient agar was incubated. No contamination of the equipment was noted, as indicated by the failure of any growth to appear on the incubated plates containing no organism.

[b] The medicament was employed in the ointment base formulations in a 1% concentration.

[c] The area of the zone of inhibition has been corrected for the area of the ointment sample, 50.28 sq. mm.

[d] Hydrophilic ointment, U. S. P. XIV.

DISCUSSION

To evaluate the surfactants with respect to their ability to release medication when tested by the

[5] Darkfield Quebec Colony Counter, American Optical Company, Buffalo, N. Y.

agar plate method, the areas of the zones of inhibition for each surfactant in the four concentrations were totaled individually for each medicament tested. For an overall evaluation, the aggregate areas for each medicament when tested in the ointment bases prepared with the individual surfactants were then totaled. The results of this evaluation—the surfactants listed in the order in which they released incorporated medication from the ointment bases—are indicated in Table II.

TABLE II.—AN EVALUATION OF THE SURFACTANTS FOR THEIR ABILITY TO RELEASE INCORPORATED MEDICATION FROM THEIR RESPECTIVE OINTMENT BASES

Mercuric Oxide	Iodine	Overall Evaluation
Brij 35	G-7596-J	G-7596-J
G-7596-J	Tween 40	Tween 40
Ethomid C/15	Ethomid C/15	Ethomid C/15
G-1045	G-1045	Brij 35
Tween 40	Myrj 49	G-1045
G-7596-P	Tween 60	G-7596-P
Tween 20	Tween 20	Tween 20
PEG. 1000 monostearate	Brij 35	Tween 60
	G-7596-P	Myrj 49
Tween 60	PEG. 1000 monostearate	PEG. 1000 monostearate
Myrj 49		

The surfactants exhibiting maximum release of medication were represented by the three chemical classifications of nonionic surfactants tested. Surfactants G-7596-J and Tween 40 are ester-type emulsifying agents, both fatty acid esters of polyethylene sorbitan. Ethomid C/15 is a fatty acid amide derivative of polyethylene glycol, while Brij 35 is a lauryl ether of polyethylene glycol.

When the ability of the nonionic surfactants to release medication was compared with sodium lauryl sulfate in hydrophilic ointment U. S. P. XIV, a striking comparison was noted. Every nonionic surfactant tested in a 1% concentration exhibited a superior ability to release incorporated medication over the hydrophilic ointment U. S. P. XIV. It was only when the concentration of the nonionic surfactant exceeded 1%, that in certain instances did hydrophilic ointment U. S. P. XIV possess a superior ability to release the incorporated medicament.

Maximum release of medication from the ointment bases was obtained when the nonionic emulsifying agents were employed in a 1% concentration in the formulation. An increase in the concentration of the emulsifying agent resulted in a subsequent decrease in the ability of the ointment base to release incorporated medication. Surfactants Tween 40, G-7596-J, Ethomid C/15, and G-7596-P produced a minimum release of medication at a 3% concentration. Beyond this surfactant concentration, an increase in release of medication was noted. With the remainder of the surfactants tested, a progressive decrease in the releasing ability of the ointment bases was noted until the concentration reached a 5% concentration. Beyond this concentration, the trend indicated an increase in the release of incorporated medication.

This observation may be explained by the fact that as the concentration of the surfactant increased the hydrophilic properties of the ointment base formulation would subsequently increase. This would result in a decreased availability of water in the ointment base for release of medication, thus resulting in a decreased release of medication from the ointment bases.

The phenomenon of the release of medication increasing after a minimum release of medication had been attained might be explained by the fact that after the surfactant had reached an optimum concentration, the surfactant would then exert an affinity for the water in the surrounding agar medium. Subsequently, this excess surfactant would diffuse out of the ointment base into the surrounding agar medium, taking with it an increased amount of the medicinal agent from the ointment base. Evidence substantiating this phenomenon was the fact that a pronounced cloudy ring was observed in the zone of inhibition probably due to the surfactant diffusing into the agar medium. This was especially noticeable when the surfactants were employed in a 7% concentration.

SUMMARY AND CONCLUSIONS

1. Ten nonionic surfactants, each employed in four concentrations were used in the preparation of hydrophilic ointment bases.

2. These ointment bases were evaluated for their ability to release incorporated mercuric oxide and iodine by the agar plate method.

3. Maximum release of medication was obtained when the ester, G-7596-J was employed in the ointment base formulation. This was followed by another ester of a similar type, Tween 40. An amide, Ethomid C/15 and an ether, Brij 35, followed in their ability to release incorporated medication.

4. Optimum release of medicament was obtained when the surfactant was employed in a 1 per cent concentration. In general, an increase in the surfactant concentration caused a decrease in the release of medication.

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Polarography of the Ethylidinium Ion of N-Ethyl-N-1-naphthylmethyl-2-chloroethylamine*,[1]

By NICHOLAS G. LORDI and JOHN E. CHRISTIAN

A study of the polarographic reduction of ethylidinium ions indicates that this method provides for a useful, rapid, and sensitive means of quantitative estimation.

OF INTEREST pharmacologically as anticarcinogens (1), adrenolytics (2), and antihistaminics (3) are 2-haloethylamines. In all three cases, the physiologically active species has been shown to be an ethylidinium ion (EDI), i. e., $RR'\overset{+}{N}CH_2CH_2$. This ion is split by reaction with anions (e.g., thiosulfate, phosphate, chloride, etc.), sulfhydryl groups, alcohols, amines, and water. Its reaction with thiosulfate has formed the basis for its quantitative estimation (4). In order to swamp the side reactions to which the EDI is subjected, a large excess of thiosulfate must be added. The residual is then titrated with standard iodine. The method is, therefore, not too sensitive.

It was thought that EDI, being a quaternary ammonium ion, should be susceptible to polarographic reduction. This presumption was verified. The thiosulfate reaction was used as an index of the concentration of EDI, the amount of thiosulfate being followed polarographically. The reaction product of the thiosulfate with EDI, $RR'NCH_2CH_2S_2O_3^-$ (EDT), was also observed to be reducible.

EXPERIMENTAL

The Sargent-Heyrovsky Recording Polarograph, Model XXI, in combination with a mercury-dropping electrode as the cathode was used throughout this work. Under open circuit and at a pressure of 23 cm. of mercury, the capillary had a drop rate of 2.9 seconds in $M/15$ phosphate buffer, pH 5.9. $m^{2/3}t^{1/6}$ was 2.56 mg.$^{2/3}$ sec.$^{-1/2}$. An H-type cell was utilized with a saturated calomel electrode. Prior to polarographic analysis, purified nitrogen was bubbled through the solutions to remove dissolved oxygen. Temperature was maintained at 25° ± 0.2° with a constant temperature bath.

Solutions of EDI were prepared by dissolving SY-14 in $M/15$ phosphate buffer of pH 5.9. The resultant cloudy solutions cleared within an hour. Measurements of current with time at a constant applied voltage of −1.4 indicated that the diffusion current did not reach its peak value until three hours after initial preparation of the solution. Since EDI was the only species present in solution which showed this type of kinetics, this constituted evidence supporting the view that it was EDI being reduced. Only catalytic hydrogen waves were obtained with solutions of SY-14 in 0.1 N KNO_3. Solutions of EDT were prepared by the addition of excess reagent sodium thiosulfate to the EDI.

The effect of pH on the half-wave potentials ($E^1/_2$) and diffusion currents (i_d in microamps) of EDI and EDT is shown in Table I. Solutions at pH 5.9 were adjusted to more alkaline and acid pH's by the addition of sodium hydroxide and phosphoric acid, respectively. It was necessary to add 0.01% gelatin to the EDT as a maximum suppressor, since, at pH 7 and above, distinct maxima obscured the waves. In contrast to the EDI, EDT was found to be stable in solution. The rate of deterioration of EDI increased with increase in pH. An uncertainty was thus introduced in the measurement of i_d and $E^1/_2$ of EDI.

The large increase in i_d of EDT with decreasing pH might be due to an increase in the concentration of its unionized form, $R'RNCH_2CH_2S_2O_3H$. EDI appears to be less sensitive to changes in pH, the greatest difference in the measured parameters occurring between pH 6 and 5.

TABLE I.—pH DEPENDENCE OF THE POLAROGRAPHIC WAVES OF EDT AND EDI

	EDT (~9 × 10^{-4} M)			EDI (~8 × 10^{-4} M)	
pH	i_d (μa)	$-E^1/_2$	pH	i_d (μa)	$-E^1/_2$
10.8	5.34	1.050	10.8	Ill-defined	
7.1	5.28	0.850	7.0	8.32	0.935
5.9	6.78	0.815	5.9	8.12	0.950
5.1	7.86	0.805	4.8	7.60	0.970
3.3	10.14	0.750	3.1	7.44	0.970

A study was also made of the effect of pH on the anodic wave of thiosulfate. Its i_d and $E^1/_2$ was found to be independent of pH. The characteristics of the wave were similar to those reported by Kolthoff and Miller (5) in 0.1 N KNO_3. i_d/C was found to be 5.11 ± 0.15 for the thiosulfate in phosphate buffer (C is the concentration in millimoles per liter). The concentration was linearly related to i_d from 0 to 2 millimolar.

A solution of EDI at pH 5.9 was made 2 mmol. in thiosulfate. When reaction was complete (1$^1/_2$ hours), the excess thiosulfate was determined polarographically and the quantity of EDT present calculated by difference. Serial dilutions were prepared and the waves recorded. i_d/C was found to be 12.36 ± 0.18 (0 to 1 mmol.). $E^1/_2$ was linearly related to the log i_d: $E^1/_2 = -0.68 - 0.127 \log i_d$. The addition of up to 5 mmol. excess thiosulfate had no effect on the characteristics of the waves.

* Received April 5, 1956, from the School of Pharmacy, Purdue University, Lafayette, Ind.

[1] N-Ethyl-N-1-naphthylmethyl-2-chloroethylamine (SY-14) is a fairly potent antihistaminic and adrenolytic. The chemical used in this work was kindly supplied by Parke, Davis and Company.

Serial dilutions of EDI (*p*H 5.9) were made at exactly fifteen-minute intervals. The wave was recorded at exactly fifteen minutes after the dilution was prepared. When 50% of the span had been transversed, an aliquot of the solution was made 5 mmol. in thiosulfate. After sufficient time had elapsed, the wave for the EDT was recorded. The deterioration of EDI by means other than reaction with thiosulfate was assumed to be negligible. i_d(EDT)/i_d(EDI) was 1.283 ± 0.058. i_d/C for EDI was then calculated to be 9.63 ± 0.48. $E^1/_2$ was approximately linearly related to log i_d for EDI: $E^1/_2 = -0.88 - 0.097 \log i_d$.

Irreversible reductions were indicated. Postulated mechanisms of reduction for both species are as follows:

$$RR'\overline{NCH_2CH_2}^+ \text{ or } RR'NCH_2CH_2S_2O^- + H^+ + 2e \rightarrow RR'NCH_2CH_2$$

The kinetics of the deterioration of EDI was followed polarographically in *M*/15 phosphate buffer. The current was measured as a function of time at a constant applied voltage midway between the half-wave potentials and the decomposition potentials of the supporting electrolytes. Plots of log i *vs.* time were linear, indicating that the deterioration of the EDI could be approximated by first order kinetics. The rate constants ($\times 10^4$) calculated from the slopes in min.$^{-1}$ were as follows: *p*H 5.9 – 14.8; *p*H 7.4 – 33.4; and *p*H 9.3 – 56.6.

Thus it appears that polarography provides a useful, rapid, and sensitive means of directly determining ethylidinium ions in solutions free of interfering substances. A study of the effect of structure on the $E^1/_2$ of EDI, especially in relation to the physiological effects of 2-haloethylamines, is in progress.

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The Condensation of 2-Mercaptothiazoline, Aliphatic Aldehydes, and Secondary Amines*

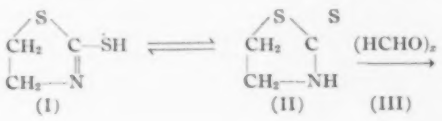
By M. P. FISHER† and W. M. LAUTER‡

A series of 2-mercaptothiazoline derivatives has been synthesized for their chemotherapeutic potential. Attempts to use aldehydes higher than formaldehyde were unsuccessful, as were attempts to use acyclic secondary amines. Infrared spectra have shown these compounds to be thiazoline-2-thiones rather than thiazolinyl sulfides.

THE INTEREST IN 2-mercaptothiazoline and its derivatives stems from the rather diverse physiological responses evoked by numerous rubber vulcanization accelerators (1, 2). Oddly, this faculty for vulcanization acceleration seems to be associated with pesticidal properties. Not only do 2-mercaptothiazoline derivatives show these actions (3), but the parent substance, itself, has exhibited considerable antithyroid activity (4, 5). Therefore a series of new thiazoline-2-thione derivatives were synthesized for their potential chemotherapeutic value.

Since there has been disagreement in the literature concerning the structures of similar compounds, synthesis was accomplished by a new modification, and, in selected cases, by literature methods also. This was done to determine whether the type of synthesis influenced the final configuration. Structure was determined spectrophotometrically.

A general reaction scheme can be formulated as follows:



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Presented to the Pharmacy Section, A.A.A.S., Atlanta meeting, December, 1955.
Abstracted from a thesis submitted by M. P. Fisher to the Graduate School of the University of Florida, in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.
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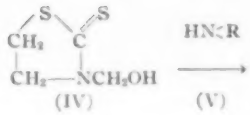
‡ Professor of Pharmaceutical Chemistry, University of Florida, Gainesville.
The authors wish to acknowledge the aid of Mr. A. R. Haskell, University of Florida, Gainesville, in performing a preliminary parasitological screening of the compounds synthesized.



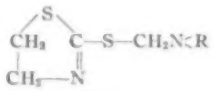
(IV) (V)

(VI)

2-Mercaptothiazoline (II) is melted and paraformaldehyde (III) added to give 3-hydroxythiazoline-2-thione (IV). This in turn is condensed with an appropriate secondary amine (V) in a suitable solvent, giving the final product (VI). The results are listed in Table I.

were entirely unsatisfactory, ending either with some of the starting materials or resinous products.

Proof of Structure.—The fact that 2-mercaptothiazoline can exist in both the thiol and thione forms suggests two possible structures for the proposed compounds, i. e., thiazoline-2-thione (VI) and 2-thiazolinyl sulfide (VII).



(VII)

The majority of the United States patent literature appears to reflect the opinion that reactions of this type favor the formation of (VII). For example, Goddin and Searle (3) condensed 2-mercaptothiazoline, formaldehyde, and morpholine to obtain a product which they label 4-morpholinomethyl-2′-thiazolinyl sulfide (VIII).

TABLE I.—THIAZOLINE-2-THIONES

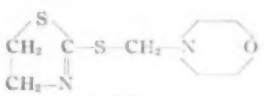
Product	Amine	M. p., °C.[a]	Yield, %[c]	Analysis Calcd.	% N and S[d] Found
XIII	Morpholine	115.5–117	84.8	N, 12.84 S, 29.38	N, 12.94 S, 29.27
XIV	Piperazine	210[b]	78.2	N, 16.08 S, 36.85	N, 16.13 S, 36.60
XV	2,5-Dimethylpiperazine	190[b]	90.5	N, 14.87 S, 34.11	N, 14.81 S, 34.48
XVI	N-Phenylpiperazine	112–113[b]	69.6	N, 14.32 S, 21.85	N, 13.92 S, 21.76
XVII	N-(*p*-Chlorophenyl)-piperazine	155–157[b]	95.2	N, 12.82 S, 19.56	N, 12.35 S, 19.50
XVIII	N-(*o*-Chlorophenyl)-piperazine	126–128[b]	81.7	N, 12.82 S, 19.56	N, 12.36 S, 19.58

[a] Uncorrected. [b] With decomposition. [c] Before purification. [d] The authors are indebted to The Goodyear Tire and Rubber Company, Akron, Ohio, for performing some of the sulfur analyses.

DISCUSSION

Scope of Reaction.—It can be noted that all the compounds in Table I are derived from alicyclic secondary amines. Also tried in combination with formaldehyde and 2-mercaptothiazoline were N-butyl cyclohexylamine, N-butyl ethanolamine, diethanolamine, N-(2-ethylhexyl) cyclohexylamine, di-(2-ethylhexyl)-amine, N-ethyl ethanolamine, diisopropanolamine, and diisopropylamine. Only with diisopropylamine was an apparently crystalline compound formed; however, attempts at purification seemed to hasten its decomposition as evidenced by a pronounced ammoniacal odor even at −20° and also by a lowering of the melting point. The other amines merely gave oily, foul-smelling liquids. It was assumed, therefore, that they either did not react as desired, or were so unstable that they decomposed immediately.

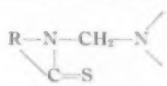
A survey of the literature has disclosed the absence of aldehydes other than formaldehyde in reactions of the type herein discussed, and unpublished reports (6) have indicated generally unsuccessful results using higher aldehydes. Therefore we attempted to condense *n*-hexaldehyde, 2-ethylhexaldehyde, pyruvic aldehyde, *n*-butyraldehyde, and 2-ethylbutyraldehyde with the amines listed above in conjunction with 2-mercaptothiazoline. The results



(VIII)

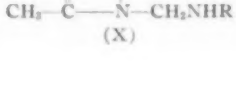
That this structure is doubtful will be shown later.

Bögemann and Zaucker (7), however, report a series of asymmetrically substituted methylene diamines of the general structure (IX), in which R represents a thiourea or five-membered heterocyclic residue, both of which may be substituted.



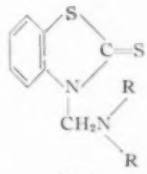
(IX)

In addition, support is lent to the thiazoline-2-thione structure from the work of Stewart and Mathes (8) on some 4,5-dimethyl-2-mercaptothiazole derivatives (X)



(X)

They cite ultraviolet evidence to substantiate the belief that the attachment of the substituent is on the nitrogen and not the sulfur. In a similar vein Kurlychek (2) also refers to ultraviolet studies on N-substituted 3-aminomethyl-2(3)-benzothiazolethiones (XI).



(XI)

Fortunately an excellent basis for comparison is available in 2-mercaptobenzothiazole. There is abundant evidence to prove that it exists predominantly in its more stable thioketone form (XII) regardless of the nature of the solvent (9–11).

(XII)

To this —N—C=S configuration has been assigned
| |

an absorption of 6.67–6.80 microns (1500–1470 cm.$^{-1}$ s.) (12).

The spectra of chloroform solutions of 2-mercaptothiazoline and 2-mercaptobenzothiazole are practically identical with respect to N—H and N—C=S, being 2.91μ (N—H) and 6.71μ (N—C=S) and 2.93μ (N—H) and 6.66–6.71μ (N—C=S), respectively. This strongly indicates that 2-mercaptothiazoline shares the same tendencies as its congener. In every case the absorption for N—C=S in these new compounds falls within the assigned frequencies. If a thiazolinyl sulfide linkage existed there would have to be a C=N; the assignment for C=N in thiazolines being 6.15–6.3μ (13). In no case does this occur. Therefore, it seems reasonable to assume that all six compounds exist in the thioketone form. The infrared spectra are shown in Figs. 1–6.

EXPERIMENTAL

General.—All the successful reactions were carried out in a standard 3-necked flask fitted with a mechanical stirrer, addition funnel, and thermometer. Cooling was accomplished by a bath of crushed ice and water. As stated above the procedure involves the formation of the methylol of 2-mercaptothiazoline followed by its condensation with a secondary amine. The methylol is formed by carefully melting the 2-mercaptothiazoline and slowly adding paraformaldehyde with slight agitation. The reaction proceeded spontaneously, additional heat being needed only where larger quantities were used. The methylol is a straw-colored liquid. It is then dissolved in a solvent in which the desired product

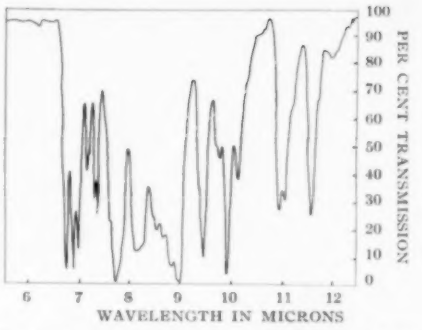


Fig. 1.—3-(4-Morpholinomethyl)-thiazoline-2-thione. Phase—chloroform.

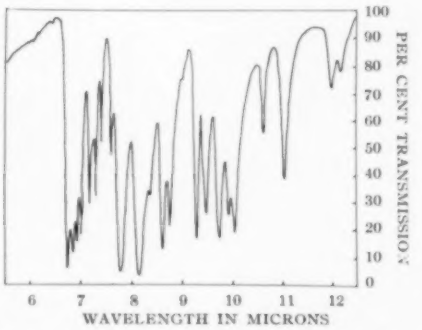


Fig. 2.—1,4-Bis-(3-thiazolinomethyl-2-thione)-piperazine. Phase—Nujol mull.

is insoluble. The solvent was chosen empirically by adding small amounts of amine to test-tube quantities of methylol dissolved in several organic solvents. For the most part the choice of solvent was not especially critical, quantity and kind merely being adjusted to produce good yields. Addition of amine was most profitably accomplished at as low a temperature as possible. Failure to do so resulted usually in discolored, odoriferous products. The reactions can also be performed without a

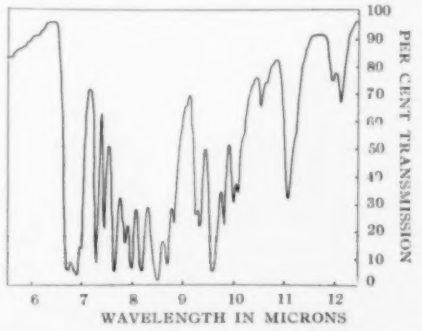


Fig. 3.—1,4-Bis-(3-thiazolinomethyl-2-thione)-2,5-dimethylpiperazine. Phase—Nujol mull.

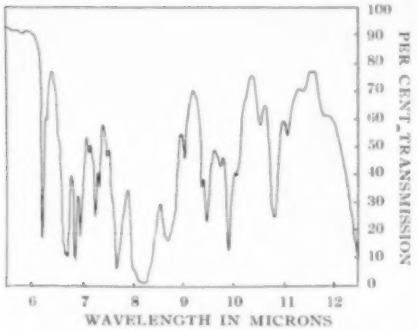


Fig. 4.—1-(3-Thiazolinomethyl-2-thione)-4-phenylpiperazine. Phase—chloroform.

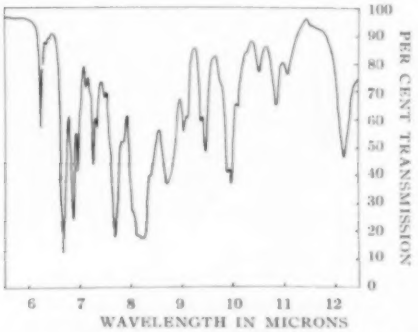


Fig. 5.—1-(3-Thiazolinomethyl-2-thione)-4-(4-chlorophenyl)piperazine. Phase—chloroform.

solvent, but yields are not as good, nor products as pure.

3-(4-Morpholinomethyl)-thiazoline-2-thione (XIII).—Eighty-nine and four tenths grams (0.74 mole) of 2-mercaptothiazoline was carefully melted and 22.5 Gm. of paraformaldehyde added with some agitation. Slight additional heat was needed to complete the reaction. The methylol was cooled to room temperature and 650 cc. of ethanol added as the solvent. Sixty-four and five-tenths grams (0.74 mole) of morpholine was added with stirring, and 50 cc. of ethanol was used to rinse down the addition funnel. A heavy, white precipitate formed soon and stirring was maintained for one-half hour. The product was recrystallized from ethanol-benzene, benzene, and finally from benzene-acetone.

This compound was also made by the methods of Goddin and Searle (3) and Stewart and Mathes (8). All three were shown to be identical by mixed melting points and infrared spectra.

1,4-Bis-(3-thiazolinomethyl-2-thione)-piperazine (XIV).—The methylol was formed from 27.6 Gm. (0.23 mole) of 2-mercaptothiazoline and 7.0 Gm. of paraformaldehyde. Acetone was used as the solvent, 200 cc. for the methylol and 300 cc. for 10.0 Gm. (0.115 mole) of anhydrous piperazine. The product is insoluble in acetone, benzene, chloroform, carbon tetrachloride, ethanol, methanol, water, tetrahydrofuran, tetrahydrofuran-benzene azeotrope, ether, dioxane, cyclohexane, and mixtures of these. It was finally recrystallized from a mixture of benzyl alcohol and ethanol (2:1). An identical product was made by the method of Stewart and Mathes (8). Again identification was made by mixed melting points and infrared spectra.

1,4-Bis-(3-thiazolinomethyl-2-thione)-2,5-dimethylpiperazine (XV).—The reactants were 29.9 Gm. (0.25 mole) of 2-mercaptothiazoline, 7.5 Gm. of paraformaldehyde, and 14.3 Gm. (0.125 mole) of 2,5-dimethylpiperazine. Two hundred cubic centimeters of acetone was used to dissolve the methylol and 250 cc. for the amine. The product is insoluble in benzene, carbon tetrachloride, ether, cyclohexanol (cold), and ethanol (cold). It was recrystallized from chloroform in which it is only slightly soluble. An identical product was made by the method of Stewart and Mathes (8), identification being accomplished by mixed melting points and infrared spectra.

1-(3-Thiazoline-2-thione)-4-phenylpiperazine (XVI).—The reactants were 20.1 Gm. (0.17 mole) of 2-mercaptothiazoline, 5.1 Gm. of paraformaldehyde, and 27.3 Gm. (0.17 mole) of N-monophenylpiperazine. Three hundred cubic centimeters of ethanol was used to dissolve the methylol and 100 cc. for the amine. The product was recrystallized from carbon tetrachloride and from ethanol.

1-(3-Thiazolinomethyl-2-thione)-4-(4-chlorophenyl)-piperazine (XVII).—The reactants were 11.9 Gm. (0.1 mole) of 2-mercaptothiazoline, 3.0 Gm. of paraformaldehyde, and 19.7 Gm. (0.1 mole) of N-(*p*-chlorophenyl)-piperazine. One hundred and twenty-five cubic centimeters of ethanol was used to dissolve the methylol and 20 cc. for the amine. The product was recrystallized from acetone and from ethanol.

1-(3-Thiazolinomethyl-2-thione)-4-(2-chlorophenyl)-piperazine (XVIII).—The same quantities of reactants were used as in the preceding paragraph, with 50 cc. of acetone as solvent for the methylol and 20 cc. for the N-(*o*-chlorophenyl)-piperazine. Fifteen minutes elapsed before a sign of reaction became evident. Shortly thereafter a dense, white precipitate formed. The product was recrystallized from acetone and from ethanol.

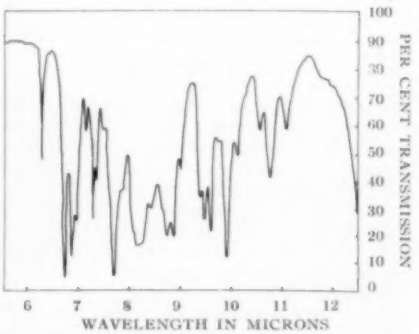


Fig. 6.—1-(3-Thiazolinomethyl-2-thione)-4-(2-chlorophenyl)piperazine. Phase—chloroform.

SUMMARY

1. An investigation of the condensation of 2-mercaptothiazoline, aliphatic aldehydes, and secondary amines has been conducted.

2. Attempts to use aldehydes higher than formaldehyde were unsuccessful. Likewise, positive results were obtained only with alicyclic or substituted-alicyclic secondary amines.

3. Five new compounds were synthesized and were shown to be thiazoline-2-thione derivatives. A sixth, reported as a thiazolinyl sulfide, was shown also to be a thiazoline-2-thione.

4. Melting points and infrared spectra are reported.

5. A preliminary parasitological investigation indicates lack of anthelmintic action on ascaris and dog hookworm.

6. Testing for rubber acceleration is now in progress.

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Determination of Carbon Disulfide in its Piperazine Compounds*

By ROGER E. BOOTH and ERIC H. JENSEN†

A rapid spectrophotometric assay for the determination of carbon disulfide in piperazine-carbon disulfide compounds has been developed. The assay has a precision of ±0.8 per cent.

THE EQUIMOLECULAR COMPLEX of piperazine and carbon disulfide has been introduced recently as a potent anthelmintic in the veterinary field. Clinically it has been shown to be nontoxic to the host, act rapidly and certainly with single dosages, show no tissue deposition and be safe for retreatment if needed (1). This compound (and related ones) may be prepared chemically by an addition reaction between piperazine (or piperazine derivatives) and an equivalent amount of carbon disulfide. The structural formula has not been definitely established. The molecular formula is $C_5H_{10}N_2S_2$.

For purity control purposes, the piperazine in the compound may be determined by acid-base titration after removal of the carbon disulfide with standard acid and heat. However, piperazine base may be an impurity in the finished product. Further, piperazine readily absorbs water and carbon dioxide from the atmosphere. These considerations indicate that the determination of piperazine alone does not ensure integrity of the drug. Therefore, from an analytical point of view an assay for carbon disulfide is necessary.

Carbon disulfide has been assayed by several modifications of the xanthate formation (2–7) and by condensation with amines (8–13). Some of these procedures are both sensitive and accurate, but are also elaborate and tedious. The ultraviolet spectrum of carbon disulfide was reported in 1925 by Bruhat and Pauthenier (14). The characteristic absorption maximum at about 320 mμ lends itself to qualitative (15) identifications. A quantitative spectrophotometric determination of carbon disulfide in carbon tetrachloride has been suggested by Andreev and Gindina (16).

In the case of the equimolecular complex of piperazine and carbon disulfide and other carbon disulfide-containing compounds, the carbon disulfide first must be liberated from the parent

* Received April 9, 1956, from the Control Division of The Upjohn Company, Kalamazoo, Mich.
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compound. It then must be trapped in some convenient way which will finally be suitable for its determination. This paper describes a method for the liberation, separation, and determination of carbon disulfide in the piperazine-carbon disulfide compound and various of its alkyl substituted derivatives. The carbon disulfide is isolated by acid hydrolysis in aqueous solution followed by extraction with chloroform. The absorbance of the chloroform solution is measured in a suitable spectrophotometer.

EXPERIMENTAL

Recommended Standard Procedure.—Transfer an accurately weighed sample equivalent to about 120 mg. of piperazine-carbon disulfide to a 200-ml. pressure bottle. Add exactly 100 ml. of chloroform U. S. P. by pipet. Add 10 ml. of 14 *N* H_2SO_4 and immediately close top tightly. Shake the charged pressure bottle one minute and then heat in a water bath at about 70° for ten minutes or more. Cool it under tap water. Place a small pledget of chloroform-washed and dried cotton in the top of a 25-ml. pipet. Draw a portion of the chloroform solution of carbon disulfide from beneath the acid layer, through the cotton into the bulb of the pipet. Invert the pipet and deliver the chloroform solution into a 1-cm. absorbance cell. Read the absorbance of the sample at 319 mμ against a portion of the same chloroform used in the sample: $[(A \times V)/(w \times a_s)] \times 100 = \%\ CS_2$, where A = absorbance of the sample at 319 mμ, w = weight of the sample in mg.; V = volume of chloroform in the sample (100 ml.); and a_s = absorbance index for CS_2 in CCl_2H at 319 mμ (concn. in Gm./L., 1-cm. cell). In this laboratory a_s was found to be 1.11.

DISCUSSION

Nature of the Method.—In this procedure the final determination of carbon disulfide depends on its absorption spectrum (see Fig. 1) in chloroform.[1] Piperazine-carbon disulfide exhibits no absorption in the indicated region. This is simply demonstrated by shaking about one-half Gm. of piperazine-carbon disulfide with 50 ml. of chloroform, filtering, and reading spectrophotometrically. The separation of liberated carbon disulfide is accomplished by only one extraction with chloroform. The distribution coefficient for carbon disulfide between chloroform and acid-water is so high that the isolation of the carbon disulfide is essentially complete in the one extraction.

The usual means of trapping a volatile liquid in an absorption train and then separating it for determination is markedly simplified by the use of the pressure bottle. The piperazine salt formed remains in the acid layer giving a clean separation simultaneously with the liberation of the carbon disulfide. The withdrawal and clarification of the shaken chloroform layer can be facilitated by the preparation of a special pipet. The bulb portion of a standard pipet is joined to the inlet of a standard two-way stopcock. The outlets of the stopcock are bent nearly parallel and lengthened. With the stopcock in one position and one "leg" of the pipet in the chloroform layer, a portion of the chloroform solution is drawn through cotton into the bulb of the pipet. The clarified chloroform solution is allowed to flow out of the second "leg" into the absorbance cell.

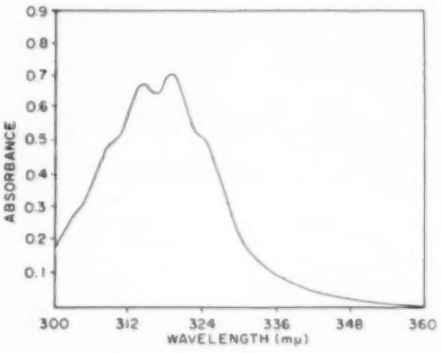

Fig. 1.—Absorption spectrum of carbon disulfide (0.6314 Gm./L. in chloroform).

Determination of the Constant a_s.—An accurately determined weight of purified carbon disulfide is dissolved in reagent grade chloroform. The absorbance is determined at the 319 mμ spectral region and the absorbance index is calculated from the value obtained at the maximum absorption. Absorbance index is defined as the absorbance in a 1-cm. cell of a solution containing 1 Gm./L.

TABLE I.—EFFECT OF ACID CONCENTRATION

		CS_2 % of Theory
Concentrated H_2SO_4		
5 ml.		101.0
5 ml.		99.3
10 ml.		98.5
	Average	99.6
14 *N* H_2SO_4		
10 ml.		98.8
10 ml.		97.6
10 ml.		98.8
	Average	98.4
1 *N* H_2SO_4		
10 ml.		99.0
10 ml.		99.3
10 ml.		97.0
	Average	98.4
0.1 *N* H_2SO_4		
15 ml.		97.9
15 ml.		98.4
15 ml.		94.4
	Average	96.9

[1] The authors gratefully acknowledge the cooperation of J. L. Johnson and J. E. Stafford of our Physics Department in obtaining the ultraviolet absorption spectra.

Effect of Acid Concentration.—The use of 14 *N* H_2SO_4 is suggested primarily from experience with ease of manipulation in this procedure. The volume of the aqueous phase can be kept low compared to the chloroform phase and there is little immediate heat given off. This allows time for making a sound closure of the pressure bottle. Other concentrations of acid which have been used are given in Table I.[2] Only 0.1 *N* H_2SO_4 gave apparently low values even though an excess was present on a calculated basis.

TABLE II.—EFFECT OF HEATING TIME AT 70°

		CS_2, % of Theory
10 Minutes		
Concentrated H_2SO_4		101.1
		99.3
		98.5
	Average	99.6
14 *N* H_2SO_4		98.8
		97.6
		98.8
	Average	98.4
1 *N* H_2SO_4		99.0
		99.3
		97.0
	Average	98.4
5 Minutes		
Concentrated H_2SO_4		101.6
14 *N* H_2SO_4		97.9
1 *N* H_2SO_4		96.1

Effect of Heating Time.—Ten minutes has been established as a suitable heating time at 70°. Three concentrations of H_2SO_4 gave good results at ten minutes at 70°. A shorter heating time (five minutes) showed lower values with the weaker acid concentrations. Examples of these findings are shown in Table II.

TABLE III.—EFFECT OF TEMPERATURE

Temperature, °C.	Time, min.	CS_2, % of Theory
100	5	97.0
100	10	Exploded when cooled
70	10	98.4
37	5	98.3
37	10	99.2
37	20	97.5
Room temperature overnight		65.2

Effect of Temperature.—Table III indicates the wide range of suitable temperatures which may be used. The 70° temperature consistently gives more uniform results and reduces the danger of explosions or leakers in the pressure bottles. Normal room temperature is not usable.

Reproducibility.—The ability to duplicate results is shown in Table IV.

TABLE IV.—REPRODUCIBILITY ON A SINGLE LOT

Sample No.	CS_2, % of Theory
1	98.8
2	97.6
3	98.8
4	96.7
5	97.8
6	96.9
7	97.9
8	97.6
9	96.7
Average	97.6
Standard deviation	± 0.8%

The average value of nine assays on a typical lot of piperazine-carbon disulfide was 97.6 ± 0.8% of the theoretical value. The correlation between carbon disulfide assays and other assays[2] on the same material is given in the following section.

Correlation of Carbon Disulfide Assays with Other Assays on Same Samples.—The data given in Table V summarize the results obtained on a few typical lots of piperazine-carbon disulfide.

TABLE V.—CORRELATION OF CARBON DISULFIDE AND OTHER ASSAYS ON THE SAME SAMPLES

Sample	Piperazine, % of Theory	*N*, % of Theory	S, % of Theory	CS_2, % of Theory
1	100.6	99.8	101.2	99.6
1	98.2	99.6	99.4	98.8
3	99.0	101.5	99.4	99.7
4	107.9	109.5	86.3	87.5
5	106.3	107.5	88.8	92.0

It should be pointed out that as the results on piperazine and total nitrogen increase the results of carbon disulfide and total sulfur decrease. This may be explained by the fact that piperazine appears to be the only impurity in the piperazine-carbon disulfide compound. Particular attention is drawn to the consistency between the carbon disulfide and sulfur assays as well as the piperazine and nitrogen assays. The correlation between the carbon disulfide determinations and the piperazine determinations on both "good" and "poor" material is clearly illustrated in the table.

Other Applications.—In addition to the compound used as an example in this discussion, the alkyl derivatives of piperazine-carbon disulfide may be assayed for carbon disulfide in this manner. Some examples are given in Table VI where the results of the carbon disulfide assay are correlated with corresponding sulfur assays. A commercially avail-

[2] The data reported in Tables I, II, III, and IV were obtained on the same batch which assayed: Piperazine = 98.2%, N = 99.4%, CS_2 = 98.9%, S = 99.5%.

[3] The authors are indebted to K. Cline of our Chemical Control Department for carrying out the piperazine, nitrogen, and sulfur assays.

able product[4] consisting of a mixture of piperazine-carbon disulfide and an inert diluent also has been assayed in this way.

TABLE VI.—ASSAYS ON SOME OTHER COMPOUNDS CONTAINING CARBON DISULFIDE

Compound	% CS_2, Theory	% CS_2, Found	CS_2, % of Theory	S, % of Theory
2-Methylpiperazine-carbon disulfide	43.2	42.6	98.7	96.9
	43.2	42.3	97.9	97.3
2,6-Dimethylpiperazine-carbon disulfide	40.0	36.3	90.1	89.6
	40.0	36.9	92.3	90.0
Parvex	10.3	10.4	101.0	...
	10.3	10.3	100.0	...

SUMMARY

A rapid method for the liberation, separation and determination of carbon disulfide in piperazine-carbon disulfide and various alkyl substituted derivatives has been developed. The carbon disulfide was liberated by acid hydrolysis, simultaneously isolated in the chloroform layer of a pressure bottle, and determined spectrophotometrically.

[4] Parvex—Registered Trademark of The Upjohn Company.

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Comparative Release of Antibiotics from Water-Miscible-Type and Grease-Type Ointment Bases*

By H. J. FLORESTANO, M. E. BAHLER, and S. F. JEFFRIES

Studies are reported on a new water-miscible base consisting essentially of polyethylene glycol diesters, in which polymyxin, bacitracin, and neomycin are stable and from which they are readily released. Using the agar cup-plate method for testing antiseptic action, differences in zones of inhibition of several species of bacteria indicated substantially greater release of each of these three antibiotics from the water-miscible base than from grease-type bases.

THE STABILITY, chemical inertness, and cheapness of petrolatum have made it one of the most favored substances for use as an ointment base. However, it is not without disadvantages. With the advent of newer chemotherapeutic agents, the concept regarding ointment vehicles has been greatly modified, with current trend toward the use of water-miscible and washable bases. A number of factors have been responsible for this change, primary among these being the differences in physico-chemical properties (e.g., diffusibility, stability) of the various medicaments incorporated, and the recognition of a need for bases with maximal penetration ability. The search for more suitable bases has been made increasingly difficult with the introduction of combined therapy, and it is here that diffusion and stability become of especial importance in the selection of an ointment base.

A new base was recently reported (1) in which both bacitracin and polymyxin were stable, and from which the antibiotics diffused much more readily than from a grease vehicle. The base[4] consisted of (*a*) liquid petrolatum, 10 Gm.; (*b*) white petrolatum, 10 Gm.; (*c*) a polyhydric

* Received February 9, 1956, from the Research Department, Pitman-Moore Company, Division of Allied Laboratories, Inc., Indianapolis, Ind.
[4] Puronic; [5] Polycin, [6] Neo-Polycin—Trade marks of Pitman-Moore Company.

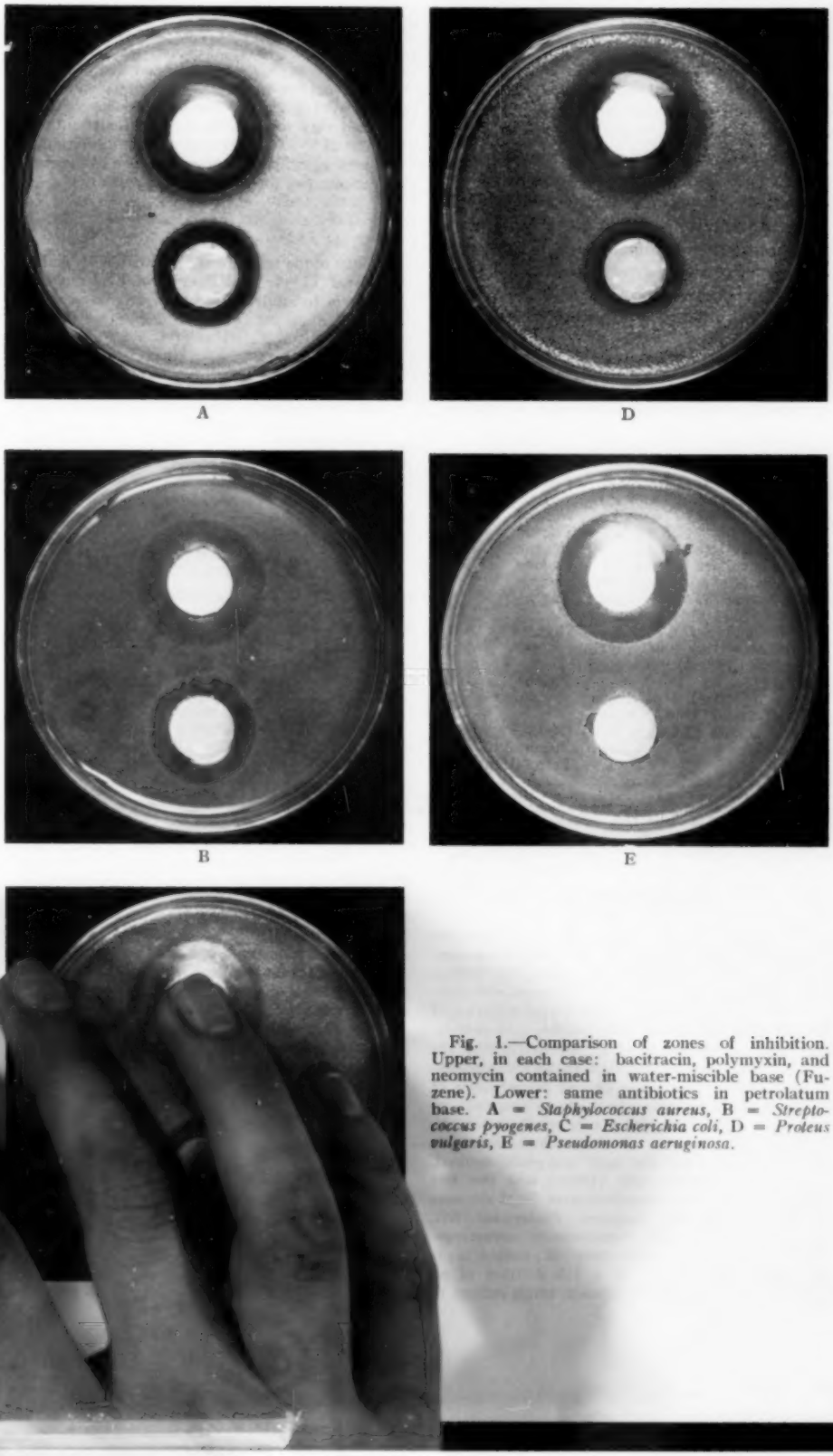

Fig. 1.—Comparison of zones of inhibition. Upper, in each case: bacitracin, polymyxin, and neomycin contained in water-miscible base (Fuzene). Lower: same antibiotics in petrolatum base. A = *Staphylococcus aureus*, B = *Streptococcus pyogenes*, C = *Escherichia coli*, D = *Proteus vulgaris*, E = *Pseudomonas aeruginosa*.

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THE STABILITY, chemical inertness, and cheapness of petrolatum have made it one of the most favored substances for use as an ointment base. However, it is not without disadvantages. With the advent of newer chemotherapeutic agents, the concept regarding ointment vehicles has been greatly modified, with current trend toward the use of water-miscible and washable bases. A number of factors have been responsible for this change, primary among these being the differences in physico-chemical properties (e.g., diffusibility, stability) of the various medicaments incorporated, and the recognition of a need for bases with maximal penetration ability. The search for more suitable bases has been made increasingly difficult with the introduction of combined therapy, and it is here that diffusion and stability become of especial importance in the selection of an ointment base.

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[1] Fuzene; [2] Polycin; [3] Neo-Polycin—Trade marks of Pitman-Moore Company

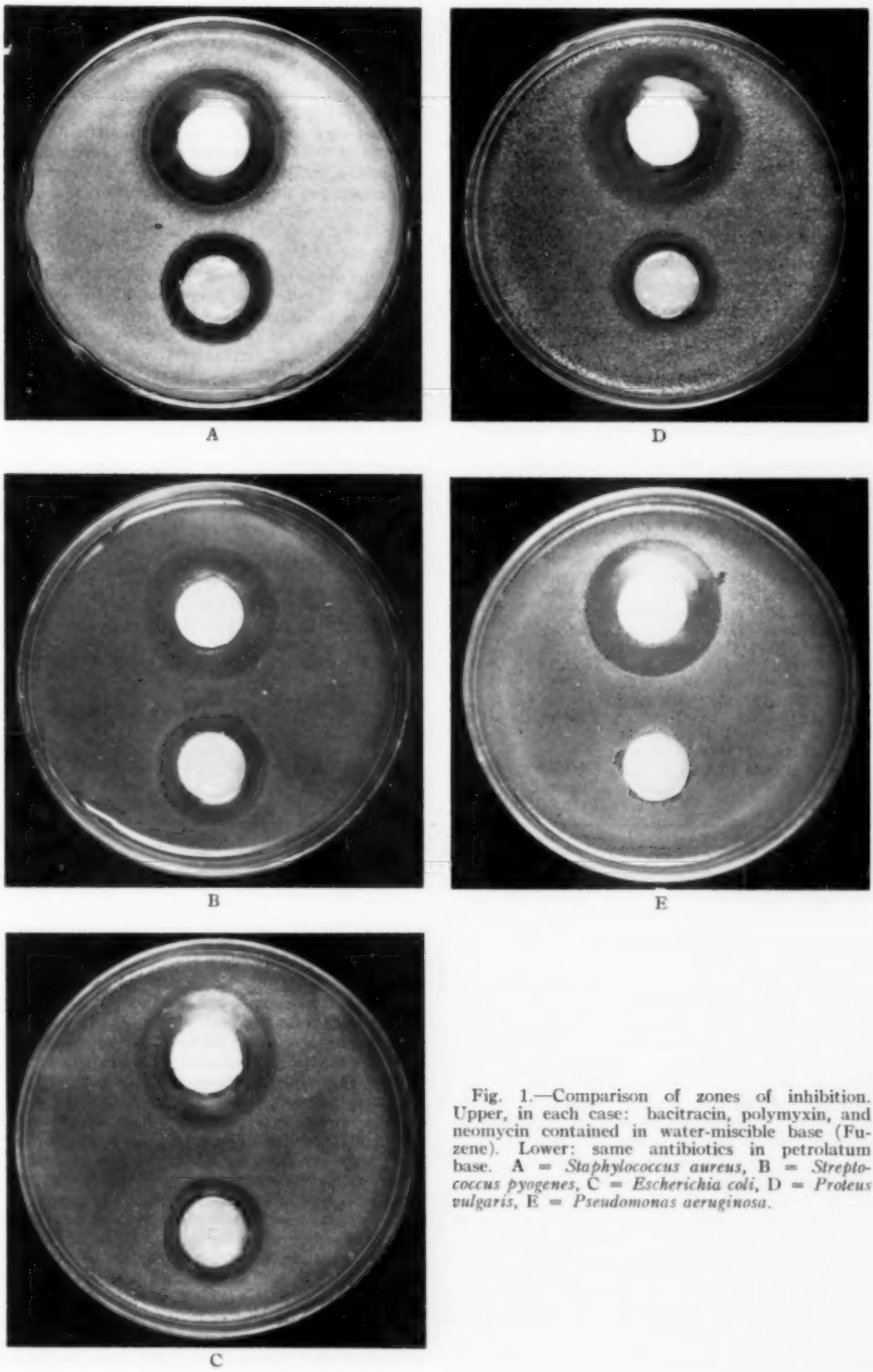

Fig. 1.—Comparison of zones of inhibition. Upper, in each case: bacitracin, polymyxin, and neomycin contained in water-miscible base (Fuzene). Lower: same antibiotics in petrolatum base. A = *Staphylococcus aureus*, B = *Streptococcus pyogenes*, C = *Escherichia coli*, D = *Proteus vulgaris*, E = *Pseudomonas aeruginosa*.

alcohol fatty acid (Glycowax S-932), 10 Gm.; (*d*) polyethylene glycol 400 di-stearate, 10 Gm.; and (*e*) polyethylene glycol 400 di-laurate, 60 Gm. A combination of bacitracin and polymyxin[2], and of bacitracin, polymyxin, and neomycin[3] in the special water-miscible base described are available for the treatment of dermatologic infections. These agents have also been used in grease-type bases (2–4). It was the purpose of this investigation, therefore, to compare the antibacterial activities of these antibiotics when incorporated in the water-miscible-type base (Fuzene) and in grease-type vehicles, employing a number of bacteria of primary significance in cutaneous infections.

EXPERIMENTAL

The respective preparations and concentrations of the antibiotics studied are given in the accompanying table. As indicated, neomycin sulfate alone in the water-miscible base (Fuzene) was compared with a commercially available preparation of the antibiotic in a grease base consisting of petrolatum, mineral oil, and lanolin. Comparison of the activity of the double and triple antibiotic combinations in Fuzene was made against preparations of the same drugs contained in a low-melting petrolatum base, the latter also commercially available. Antibacterial potencies of the various ointments were determined by the agar cup-plate method. Brain heart infusion agar (Difco) was the test medium, and the test organisms were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. Twenty-five cubic centimeters of melted agar inoculated with 0.5 cc. of a 1:5 dilution of an eighteen-hour brain heart infusion broth culture of test organism was added to each plate. Preparations were assayed in plain medium and in medium containing 10% normal horse serum. A fixed amount (400 mg.) of each ointment was placed into cups 1.5 cm. in diameter. All preparations were tested in triplicate. Plates were covered with unglazed clay tops and incubated for twenty-four hours at 37°. The extent of growth inhibition was measured in millimeters from the edge of the cup to the periphery of the zone (radius segment).

RESULTS

Results of the tests are summarized in Table I and illustrated in Fig. 1. Failure of bacitracin and polymyxin to inhibit *Proteus vulgaris* was expected since these agents were ineffective against this organism in concentrations greater than 500 u./ml.

TABLE I.—COMPARATIVE ACTIVITIES OF ANTIBIOTICS INCORPORATED IN VARIOUS OINTMENT BASES

Preparation, Antibiotics (Concn. per Gm. of Ointment)	Base	Clear Zones of Inhibition in Mm.[a]							
		Proteus vulgaris		*Pseudomonas aeruginosa*		*E. coli*	*Staph. aureus*		*Strep. pyogenes*
		S[b]	NS	S	NS	S	S	NS	S
Bacitracin (400 u.)	Fuzene	0	0	...	...	...	...	...	...
Polymyxin B sulfate (8000 u.)	Fuzene	0	0	...	...	...	...	...	...
Neomycin sulfate (0.75 mg.)	Fuzene	5.0	5.3	...	...	...	...	...	...
Neomycin sulfate (1.5 mg.)	Fuzene	5.5	6.5	...	...	...	...	...	...
Neomycin sulfate (4.5 mg.)	Fuzene	6.8	7.4	...	...	...	...	...	...
Neomycin sulfate (5.0 mg.)	Grease[c]	3.5	4.8	<0.5[e]	2.3[e]	...	5.5	4.7	...
Bacitracin (400 u.) + polymyxin B sulfate (8000 u.)	Fuzene	0	0	3.7	4.4	...	5.0	5.4	...
Bacitracin (500 u.) + polymyxin B sulfate (10,000 u.)	Grease[d]	0	0	1.8	2.5	...	<1.0[e]	<1.0[e]	...
Bacitracin (400 u.) + polymyxin B sulfate (8000 u.) + neomycin sulfate (3.0 mg., base)	Fuzene	10.7	...	8.0	...	3.0	9.0	...	8.3
Bacitracin (400 u.) + polymyxin B sulfate (5000 u.) + neomycin sulfate (3.5 mg., base)	Grease[d]	4.5	...	0.7	...	1.3	5.2	...	5.8
Control	Fuzene	0	0	0	0	0	0	0	0
Control	Petrolatum	0	0	0	0	0	0	0	0

[a] Figures represent averages of triplicate determinations, respectively.
[b] S = agar with 10% normal horse serum; NS = plain agar.
[c] Base consisted of petrolatum, mineral oil, and lanolin.
[d] Low-melting petrolatum.
[e] Partial inhibition.

and 8000 u./ml., respectively, when tested by the serial twofold dilution method. The organism, however, is susceptible to neomycin, and a high release of this antibiotic from the water-miscible base was apparent from the wide zones of inhibition produced by as little as 0.75 mg. of the sulfate per Gm. of ointment. In contrast, a concentration of 5.0 mg. of neomycin sulfate was required per Gm. of grease base to produce comparable effects, indicating a lower degree of release from this type of vehicle. Regardless of base, two kinds of inhibition zones were produced by neomycin against *Proteus vulgaris:* a clear zone immediately surrounding the cup, and a smaller zone peripheral to the clear area, showing partial inhibition only. Similar dual zoning was observed when the triple combinations containing neomycin were tested against *Escherichia coli*, although with this organism the zone of partial inhibition was the larger. Such secondary zones often result with the agar cup-plate technique. Worthy of further mention, however, was an additional type of zoning observed when neomycin

was tested against *Staphylococcus aureus* and when the triple antibiotic preparations were employed against the *Staphylococcus* and *Proteus vulgaris*. In these instances, an appreciably wider zone of inhibition resulted at the surface of the agar than at the bottom, giving the outer boundary of the clear zone a sloping appearance (Fig. 1, A, D).

With bacitracin effective essentially against gram-positive organisms and polymyxin highly specific in action against *Pseudomonas aeruginosa*, the results obtained with combinations of these agents indicated that both bacitracin and polymyxin were also released to a greater extent from the water-miscible base than from petrolatum. In tests with the triple antibiotic mixtures, particularly notable were the differences in activity shown by the two preparations against *Proteus vulgaris* and *Pseudomonas aeruginosa*. Incorporated in the water-miscible base, an inhibition zone of 10.7 mm. (surface width) was produced against *Proteus vulgaris*, compared to a zone of 4.5 mm. for the petrolatum ointment. Against *Pseudomonas aeruginosa*, a zone of 8.0 mm. resulted with the water-miscible preparation against one of only 0.7 mm. occurring with the triple mixture in the grease base. Since neomycin is highly effective against *Proteus* and possesses, in addition, significant activity against *Pseudomonas*, these data show a similarity of neomycin to polymyxin with respect to low diffusion from the grease base.

Neither the water-miscible base nor petrolatum *per se* showed any inhibitory action on the organisms tested. Presence of serum in the medium appeared to have only a slight effect on antibiotic activity.

DISCUSSION

It is believed that reliable information on the potential clinical efficacy of topical preparations can be obtained by the *in vitro* technique employed, since the test simulates to a certain extent actual conditions of use. With the agar cup-plate procedure it must be realized that zones of inhibition are not necessarily absolute indications of relative drug activity, since these are, to a large extent, functions of the degree of diffusion of antimicrobial agents into the surrounding medium. On the other hand, the gradient of penetration through the agar is, in turn, dependent upon the amount of substance released from the vehicle employed. In the present investigation, results showed that antibiotic release from the respective bases was the factor responsible for the wide differences in inhibition zones. The demonstration of poor release of antibiotics from grease-type bases is in agreement with findings of other investigators (1, 2). The significance of these data may well serve to explain the low clinical efficacy of other agents when incorporated in grease bases. Ammoniated mercury may be ineffective in petrolatum (5). Sulfathiazole has been shown to be more effective in an oil-in-water emulsion base than in an all-grease base (6). Chloromycetin in a water-miscible base was found to have definite advantages over a petrolatum preparation in acute cases of pyogenic dermatoses (7).

Laboratory and clinical data (1, 8, 9) obtained with the combination of bacitracin and polymyxin B sulfate in the water-miscible base described have established it as a unique ointment vehicle. These antibiotics as well as neomycin sulfate are not only stable in the base but their ready release permits maximal drug concentrations at the site of infection. Penetration into tissue exudates is further enhanced since the base is miscible with blood, serum, and pus.

SUMMARY

1. The antibacterial properties of bacitracin, polymyxin B sulfate, and neomycin sulfate when incorporated in a water-miscible-type base, consisting essentially of polyethylene glycol diesters, and in grease-type bases have been compared by the agar cup-plate method.

2. Wide differences in zones of inhibition of five species of bacteria involved in cutaneous infections indicated substantially greater release of each of the antibiotics from the water-miscible base than from the grease-type bases.

3. The advantages of the polyethylene glycol diester-base over grease-type vehicles are discussed.

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The Pharmaceutics of Coating Tablets by Compression*

By JOHN WINDHEUSER and JACK COOPER

The advantages of coating tablets by compression rather than in standard rotating pans are discussed. Results obtained with the Manesty DryCota are sufficiently favorable to arouse further interest. Assured presence of cores is obtained by electro-mechanical means. Formulation problems are analyzed and typical granulations described. Dry coated tablets appear to be more stable to elevated temperature and humidity than pan coated tablets.

DRY COATING of tablets by compression is a "new" technique which has aroused a great deal of interest throughout the pharmaceutical industry. This is a process by which a tablet may be coated with a dry material by compression. The finished tablet consists of a core around which a coating has been compressed. The nature of the coating and its thickness may vary as the conditions demand (Fig. 1).

Fig. 1.—Chlorisondamine (Ecolid) rotocotes®.

It is surprising, after being exposed to claims and counterclaims of various machine manufacturers, to find that the concept of dry coating is not new but dates back to at least 1895 when Parker J. Noyes was granted a British patent (1) for a special tablet machine. In 1917 a U. S. patent was granted for a double rotary machine which claimed a process "for mechanically applying and compressing a powder, as sugar, upon the faces of previously prepared tablets" (2). Another American inventor was granted a patent (3) which indicated that he was aware of the necessity of obtaining proper centering of the core within the tablet. The patent states that concave punches tend to center the core in the die, and if this does not take place automatically, a centering device can be used.

The evolution and development of modern dry coating machinery is reviewed by Mitchell (4) Robinson (5), and Tsevdos (6) who thereby also clarify the part played by their respective companies, i. e., Manesty, Killian, and Stokes.

Up to the present time the published literature dealing with dry coating is mainly of a historical or mechanical nature. It is the purpose of this paper to present some experiences, stability data, and comments derived from the preparation of dry coated tablets on a research and production basis.

COATING PROCESSES

Pan Coating.—Before discussing dry coating further, it is best to review briefly the process of pan coating as it is done today. This process basically consists of subcoating of the nuclei, smooth coating, color or finished coating, and polishing. The general procedure is to apply a subcoat to the tablets to round out the sharp edges and to build them up to the desired shape. This is done by applying heavy syrups to the tablets and dusting with powders such as flour. The tablets are then smoothed by the application of sugar syrups until the desired size and smoothness have been achieved. The tablets are finished either with plain syrup or with colored syrups. After completing the color coating, the tablets are polished by the application of waxes either in solution or in the dry state. It is obvious that this process is not ideal as it is time consum-

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ing, requires the exercise of skilled judgment by the operator, and also presents other difficulties due to the presence of moisture during the coating and possibly in the finished tablet. Substances which are affected by moisture must be protected during the coating process by moisture repellent coatings which in turn tend to prolong unduly the disintegration time of the finished tablet.

Dry Coated Tablets.—The equipment available at present for the preparation of dry coated tablets falls into two basic types. The first type coats cores which have previously been prepared on other tablet machines. The precompressed cores are placed in a vibrating hopper which feeds the nuclei to a transfer system which then deposits the tablet onto a bed of coating granules. As the turret rotates the die is completely filled with granules. The compression stroke follows and the coated tablet is ejected in the usual manner. The second type consists of a dual unit which compresses the core and coats it in one continuous cycle. The Manesty DryCota machine is a unit of this type and all tablets discussed in this paper were prepared on it.

This machine consists of two D3 type rotary tablet machines connected by a transfer system. The cores are prepared on the first turret and are transferred to the second turret and coated. As the cores are being transferred to the coating turret, they pass over a vacuum suction device which dedusts them and also prevents the transfer of any of the core granulation into the coating granulation, thereby preventing any contamination which would result in mottled or discolored finished tablets. After the tablet passes over the dedusting device, it is deposited in the coating die, coated, compressed, and ejected as in the type 1 machine. During the operation of the machine, samples of the core may be taken without stopping the machine thus allowing the operator to check the weight of the core at any time without interrupting the cycle.

One of the major difficulties confronting the manufacturers of these tablet machines was the development of controls which would insure the presence of a nucleus in every finished tablet. Various means were recommended such as X-raying the finished tablets or subjecting the tablet to pressure, assuming that a coreless tablet was softer than a perfect tablet. These methods were rejected as being either too expensive or not effective. It was decided to develop in-process controls which would determine whether a core was being fed to the coating turret and deposited in the die and retained there. This system is operated by microswitches which are activated by the plunger cap of the transfer cup. When a core is present in the cup, the pin is raised. This is checked by a microswitch (Fig. 2). Should the cap be down, signifying the absence of a core, the microswitch would activate a timed-delay relay which would in turn cause the resulting blank tablet to be rejected. Another microswitch is set to check that the core delivered to the coating turret is retained in the die. During the return cycle of the transfer system, the cap of the plunger should be down (Figs. 3 and 4).

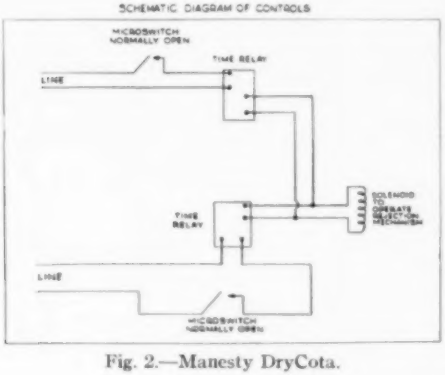

Fig. 2.—Manesty DryCota.

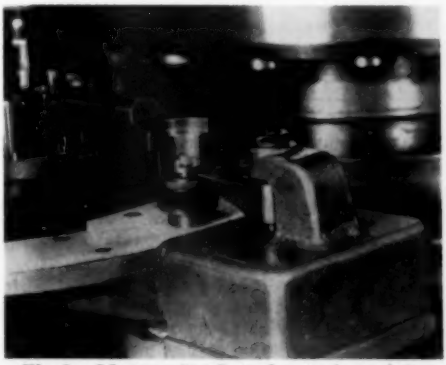

Fig. 3.—Manesty DryCota, front microswitch.

Fig. 4.—Manesty DryCota, rear microswitch.

But if a cap is raised, indicating that a core had been retained in the cup, the microswitch would react and trigger the rejection mechanism as previously described. By this means we have been able to control our tablets to a point where all rejects are segregated.

Problems Encountered.—The pharmaceutical problems encountered in the dry coating of tablets are similar to those encountered in tablet manufacture and in conventional pan coating. The tablets must not stick to the punches, bind, or cap. The

coating must be uniformly applied on all sides and must present a homogeneous color appearance. In preparing tablets a major problem encountered is the bonding or rather the lack of bonding of the core to the outer coating. The problem of bonding of two different granulations was approached in two ways: pharmaceutical and physical. By virtue of many trial and error experiments, it was found that the incorporation of a waxy substance into both the core and coating granulations greatly increased bonding (Table I).

TABLE I.—A TYPICAL COATING GRANULATION

Lactose	73%
Tragacanth	2%
Confectioners sugar	15%
Lubricant	1–5%
Talcum	5%
Color	q. s.
Distilled water	q. s.
Alcohol	q. s.

Another problem is the centering of the core within the coating granulation. In this respect the granule size of the coating granulation is critical. It must be fine enough to flow smoothly around the core and yet not contain too many fines. The failure of a granulation to flow smoothly around the core will result in a preponderance of tablets with off-center cores. Consideration must also be given to the hardness of the coating granules. If the granules are excessively hard, the coating "bed" in the die offers little resistance to the centrifugal force applied to the core by rotation of the turret and off-centering takes places. An experiment was conducted to determine the effect of various machine speeds on tablets prepared with a coating granulation with poor flow characteristics (Table II). The same experiment was run using a granulation with good flow characteristics and found to yield perfect tablets at all speeds.

TABLE II.—EFFECT OF MACHINE SPEEDS ON TABLETS PREPARED WITH A COATING GRANULATION NOT HAVING GOOD FLOWING CHARACTERISTICS

Tab./Hr.	Good[a]	Fair[b]	Bad[c]
21,600	17	15	18
20,160	21	15	14
19,200	29	18	5
18,240	38	9	3
16,800	30	20	0
15,840	37	13	0
14,880	40	9	1
12,480	44	6	0
10,500	47	3	0

[a] Centered; [b] Slightly off center; [c] Off center, rejects.

The nature of the core granulation is as important as that of the coating granulation. The granules comprising the core granulation generally should be of a larger particle size than those of the coating, since it is desirable to prepare a core which is porous in nature. The coating then penetrates the interstices giving better bonding than with a smooth hard core. Our experimental results indicate that a granulation having a mesh size of from 14 to 16 is optimal for most core granulations (Table III).

TABLE III.—A TYPICAL CORE GRANULATION

Active ingredient	q. s.
Ammonium calcium alginate (Keltose®)	2%
Lactose	q.s. to 100%
Carbowax® 6000	2½%
Alcohol U. S. P.	q. s.
Distilled water	q. s.
Corn starch	5%
Stearic acid	2%

Proper control of the hardness of the core prior to coating is essential. Linde (7) has found that the core should usually be compressed very soft. We have confirmed this finding by noting that a core

TABLE IV.—TEMPERATURE EFFECTS ON CHLORISONDAMINE TABLETS

			Weeks				
°C.	Dry Coated	Control	1	2	3	4	8
Chlorisondamine, 25 mg.							
10	D.T.[a]	7	8	8	8	9	7
10	H.[b]	10	10	9	8	9	9
25	D.T.	7	8	9	8	8	8
25	H.	10	10	10	8	8	7
40	D.T.	7	8	8	8	8	7
40	H.	10	12	12	8	8	7
50	D.T.	7	6	10	9	8	7
50	H.	10	10	11	9	8	7
Chlorisondamine, 50 mg.							
10	D.T.	11	11	11	12	11	11
10	H.	18	17	17	16	16	18
25	D.T.	11	10	11	18	11	12
25	H.	18	17	17	16	16	15
40	D.T.	11	13	11	11	13	11
40	H.	18	15	19	15	15	15
50	D.T.	11	14	14	12	12	10
50	H.	18	18	17	15	13	13

[a] Disintegration time in minutes; [b] Hardness (Strong Cobb).

with a Strong-Cobb hardness up to 4 Kg./in.^{2} is optimal. This core hardness was found to yield the most satisfactory tablet although in one case it was found that a core with a hardness of 6 gave a far superior tablet than one with a lesser hardness. It is probable that the optimal hardness of core and finished tablet must be determined for each specific case.

The appearance, hardness, and disintegration times of various dry coated tablets prepared in our laboratories showed little change when exposed to temperatures of 10°, 25°, 40°, and 50° for periods as long as eight weeks. Typical results obtained with dry coated chlorisondamine tablets are shown in Table IV.

Hydralazine hydrochloride (Apresoline HCl–Ciba) tablets, both pan coated and dry coated, were exposed to 84% relative humidity at 25° and checked for color change, since it is known that the standard pan coated hydralazine tablet turns from blue to green when exposed to extremely high humidity for any length of time. The tablets were checked daily. After three days the pan coated tablets were green. The dry coated tablets retained their original blue color for more than two months.

The effect of 95% relative humidity for forty-eight hours on various pan coated and dry coated tablets was determined and the results are described in Table V.

TABLE V.—THE EFFECT OF NINETY-FIVE PER CENT RELATIVE HUMIDITY FOR FORTY-EIGHT HOURS ON PAN COATED AND DRY COATED TABLETS

Product	Moisture Gain, %	Remarks
Adiphenine (Trasentine®)–phenobarbibital (Pan coated)	17.6	Coat completely dissolved. Tablet 100% deteriorated.
Adiphenine-phenobarbital (Dry coated)	9.05	Tablet soft but retains shape and can be handled.
Hydralazine, 25 mg. (Pan coated)	16.8	Color changed to green. Coat completely dissolved. Tablet 100% deteriorated.
Hydralazine, 25 mg. (Dry coated)	8.08	Color blue. Tablet soft, but retains shape and can be handled.
Chlorisondamine (Ecolid®), 25 mg. (Dry coated)	9.74	No color change. Soft, but can be handled.
Chlorisondamine 50 mg. (Dry coated)	8.27	No color change. Soft, but can be handled.
Chlorisondamine 100 mg. (Dry coated)	8.79	No color change. Soft, but can be handled.

These preliminary stability studies indicate that dry coated tablets can be expected to be more resistant to the influence of moisture than pan coated tablets and that in most cases they will not be adversely affected by temperatures from 10° to 50°.

DISCUSSION

The advantages of the dry coating process are multiple. The first and most obvious is the lack of water in the coating stage. This facilitates the coating of tablets which would ordinarily be impossible to coat under the standard conditions. Substances such as penicillin, aspirin, and others which are hygroscopic or react with water are difficult to coat by the standard pan coating process. Dry coating now provides a means of preparing a stable dosage form of these compounds. Dry coating offers the advantage of allowing the combination of incompatible ingredients in one tablet by placing one in the core and the other in the coating. All of the possible uses of dry coating have not been explored, but such applications as prolonged action tablets, coatings with special resistance to tropical environment, and compressed enteric coatings will undoubtedly be investigated (Fig. 5).

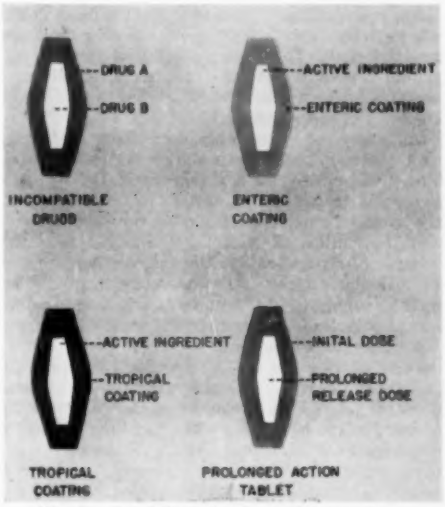

Fig. 5.—Special applications of dry coating.

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Calculations on the Countercurrent Separation of Weak Acids and Bases*

By HYMAN MITCHNER† and LLOYD M. PARKS

A simple expression has been derived for predicting the separation to be obtained from the countercurrent distribution of binary mixtures of ideally behaving weak acids or bases under conditions for optimum separation.

With the advent of countercurrent distribution there has been considerable interest shown in the partitioning of weak acids and bases between organic and immiscible aqueous phases, particularly in the isolation and purification of therapeutic agents (1–3). It was early seen that the alteration of the hydrogen ion concentration of a buffered aqueous solution exerted a pronounced effect on the observed partition coefficients (4, 5). If the substances to be partitioned behave ideally, i. e., possess a linear partition isotherm, the partitioning can be controlled according to a very simple linear relationship. This control of the distribution allows one to adjust conditions to those for optimum separation (6, 7). Under conditions for the optimum separation of two substances, simple and useful relationships have been derived to predict the efficiency of separation and purity.

A number of methods for correlating separation and partition coefficients have been suggested. Two of the most general are the method of Weisiger (8) which relates the separation to be obtained to the spread between the peaks of two components; and the method of Nichols (9) which relates the "degree of separation" to the intersecting point of the distribution curves for two components. The method suggested in this paper is, where applicable, not as complex as either. In the separation of the alkaloids veratridine and cevadine by countercurrent distribution (10) it has given good results and its simplicity, under the restrictive conditions given, suggests that it may be of use to others interested in the countercurrent separation of ideally behaving weak acids and bases. Although the expressions are mathematically rigorous, they are not necessarily confined to exact conditions. They are intended more as a guide to give an approximate numerical value for the number of transfers needed to effect a given separation, in terms of the relative amounts of the two substances to be separated, and for a predetermined purity of one or both of the components.

Determination of Optimum Conditions

pH and Partition Coefficient.—Craig (4) derived expressions for the variation of the partition coefficients of bases with pH. These expressions were analogously applied to weak acids by Golumbic, Orchin, and Weller (11). For weak acids, the observed partition coefficient K is given by

$$\log K = -p\mathrm{H} + pK_a + \log K' \qquad (1)$$

where K_a = ionization constant of the acid and K' is the partition coefficient of the unionized acid. The analogous expression for bases is

$$\log K = p\mathrm{H} + pK_b + \log K' - p\mathrm{K}_w \qquad (2)$$

where K_b = ionization constant of the base, K' is the partition coefficient of the unionized base and K_w is the ionization constant for water. At constant temperature the quantities K_a, K_b, K' (both for acids and bases), and K_w are constant. Equation (1) at constant temperature can be expressed as

$$\log K = -p\mathrm{H} + A \qquad (3)$$

where $A = pK_a + \log K'$. Similarly, Eq. 2 can be expressed as

$$\log K = p\mathrm{H} + B \qquad (4)$$

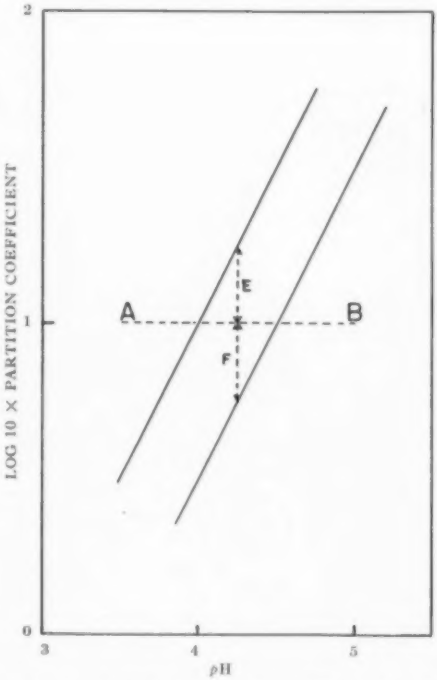

Fig. 1.—A typical log $10K$ *vs.* pH relationship between two alkaloids possessing linear partition isotherms.

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† WARF Research Assistant, 1954–56.

where $B = pK_b + \log K' - pK_w$. Both equations represent straight line relationships of slopes -1 and $+1$, respectively.

pH for Optimum Separation.—Conditions for optimum separation occur (assuming equal volumes of upper and lower phases) when $\sqrt{K_1K_2} = 1$ or this can be taken as when $K_1 = 1/K_2$ (6, 7). If the two substances to be separated are weak bases possessing linear partition isotherms, then Fig. 1 represents a possible log K *vs.* pH relationship. It can easily be shown that $K_1 = 1/K_2$ when the distance E perpendicular to AB, from $K = 1$, is equal to the opposite perpendicular F.

Separation and Purity Considerations.—When more than twenty transfers are run in a Craig type countercurrent distribution the fraction of material in any tube, r, is given by the expression

$$T_{n,r} = \frac{1}{\sqrt{2\pi nXY}}\, e^{-a^2/2nXY} \qquad (5)$$

where n = number of transfers; a = number of tubes from the peak tube, the position of the peak tube is given by $r_{max.} = nX$; $X = K/K+1$, where K is the observed partition coefficient, $Y = 1/K + 1$. Equation (5) is that of the normal curve of error of statistics and $\sqrt{nXY}$ is θ, the average deviation from the mean of the curve and where θ also determines the width of the theoretical curve (8).

If conditions are those for the optimum separation ($K_1 = 1/K_2$) of two substances, I and II, it can readily be shown that the band widths of both substances are equal, i. e., $X_1Y_1 = X_2Y_2$. The amount of material in tube nX_1 is designated $T_{1\ max.}$, that in tube nX_2 is designated $T_{2\ max.}$ such that $T_{1\ max.} = D_1T_{0\ max.}$ and $T_{2\ max.} = D_2T_{0\ max.}$ where D_1 and D_2 are constants to convert the fraction of material in the peak tubes of I and II (fraction in peaks are equal) to experimental amounts. The corresponding amounts of I and II in tube $n/2$ is designated as T_1 and T_2, respectively (see Fig. 2). The distances A_1 and A_2 between the peak tube of I to tube $n/2$ and the peak tube of II to $n/2$ are given by

$$A_1 = n/2 - nX_1 = \frac{n}{2}(1 - 2X_1) \qquad (6)$$

$$A_2 = nX_2 - n/2 = \frac{n}{2}(2X_2 - 1) \qquad (7)$$

The amount of material I in tube $n/2$ is given by

$$T_1 = \frac{D_1}{\sqrt{2\pi nX_1Y_1}}\, e^{\frac{-a(1-2X_1)^2}{8X_1Y_1}}$$

Similarly the amount of material II in tube $n/2$ is given by

$$T_2 = \frac{D_2}{\sqrt{2\pi nX_2Y_2}}\, e^{\frac{-a(2X_2-1)^2}{8X_2Y_2}} \qquad (9)$$

However, when $K_1 = 1/K_2$,

$$\frac{(1 - 2X_1)^2}{8X_1Y_1} = \frac{(2X_2 - 1)^2}{8X_2Y_2} = C.$$

If either of these expressions is expressed in terms of K, such that K is consistently either K_1 or K_2, then

$$C = \frac{(K - 1)^2}{8K} \qquad (10)$$

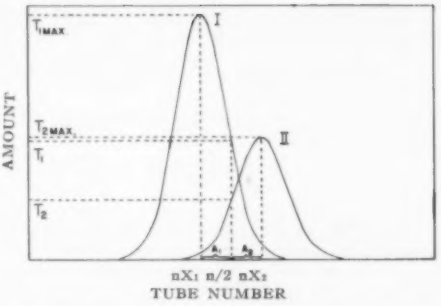

Fig. 2.—A theoretical countercurrent separation of two ideally behaving substances, I and II, under conditions for optimum separation, but where there is twice as much of I as of II.

As

$$\frac{1}{\sqrt{2\pi nX_1Y_1}} = \frac{1}{\sqrt{2\pi nX_2Y_2}} = T_{0\ max.},$$

Eqs. 8 and 9 become

$$T_1 = T_{1\ max.}\, e^{-Cn} \qquad (11)$$

$$T_2 = T_{2\ max.} e^{-Cn} \qquad (12)$$

$$T_1 + T_2 = (T_{1\ max.} + T_{2\ max.}) e^{-Cn}$$

$T_1 + T_2$ represent the total amount of material in tube $n/2$ and is taken as $T_{n/2}$. Therefore

$$n = 2.3 \log \frac{T_{1\ max.} + T_{2\ max.}}{T_{n/2}} \Big/ C \qquad (13)$$

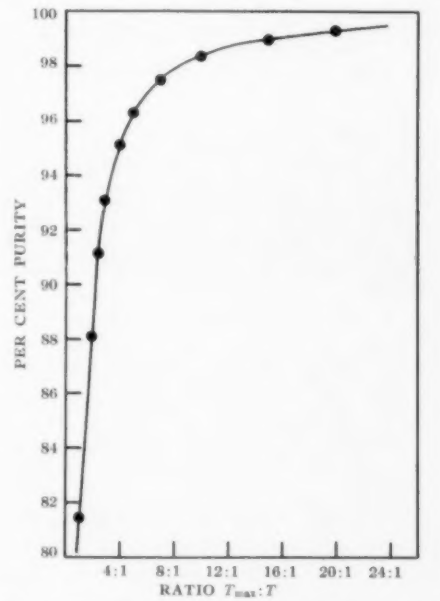

Fig. 3—Increase in per cent purity with increase in R ($T_{max}:T$) ratios.

If Equations (11) and (12) are solved independently for n it becomes apparent that

$$\frac{T_{1\,max.}}{T_1} = \frac{T_{2\,max.}}{T_2} = \frac{T_{1\,max.} + T_{2\,max.}}{T_{n/2}} \quad (14)$$

For generality these ratios are referred to as R and the general equation would be

$$n = \frac{2.3 \log R}{C} \quad (15)$$

By setting any of the ratios R from Eq. 14 to some predetermined value it is now possible to easily calculate the number of transfers necessary to attain this ratio, e. g., if $(T_{1\,max.} + T_{2\,max.})/T_{n/2} = 10:1$, then $n = 2.3/C$.

The greater the ratio, the better is the separation obtained. If the amounts of the two substances present are about equal, a ratio of 20:1 would give quite pure material for both substances—tube $n/2$ is taken as the dividing tube when collecting.[1]

The purity, of course, is dependent on the relative concentrations of the two substances, i. e., for any given number of transfers, the material present in the largest amount will have the greatest purity. By use of the probability function tables for the determination of area under a normal distribution curve (9), the increase in percentage purity with increase in the ratio $T_{max.}/T$ can be calculated (see Table I). A plot of this function is given in Fig. 3.

TABLE I.—INCREASE IN PURITY WITH INCREASING $T_{max.}/T$

$T_{max.}/T$	Area, %
1.5:1	81.43
1.75:1	85.49
2.0:1	88.04
5.0:1	96.33
10.0:1	98.38
20.0:1	99.27

[1] It is difficult to prepare a buffer solution to an exact pH and a small variation in pH creates a more marked effect on the partition coefficient. The degree of separation will still be close to that predicted. However, tube $n/2$ no longer corresponds to the same tube in the theoretical expressions. Futhermore, in actual practice even for an exact pH, the separation would probably be effected not at tube $n/2$, but at the tube representing the lowest total material content. This latter also is the case where the pH might vary slightly. It has been found that the values obtained even under slightly nonideal conditions still correspond well with those values predicted.

If the amounts of I and II are equal, the area represents the percentage of purity. However, for unequal amounts a correction factor must be introduced. If substance I is taken as being present in the greater quantity, then the percentage purity of I is greater than the percentage area. The purity can be calculated by using the factor $F = T_{2\,max.}/T_{1max.}$ For example, if the distribution is carried out to give a $T_{max.}/T$ ratio of 5:1 when there is twice as much of I as of II, then area of I up to $n/2 = 96.33$. Corresponding area of II included $= F \times (100 - 96.33) = 1.84$, % purity of I $= 96.33/(96.33 + 1.84) = 98.12$. Similarly with II: area of II after $n/2 = 96.33$. Corresponding area of I included $= 1/F \times (100 - 96.33) = 7.34$, % purity of II $= 96.33/(96.33 + 7.34) = 92.99$.

SUMMARY

It is known that the partitioning of weakly acidic or basic organic systems can be altered according to a simple linear relationship if the partitioned substances possess a linear partition isotherm. This control makes it possible to set up conditions for the optimum separation of two such substances by countercurrent distribution. Under such conditions it is possible to predict in a simple manner the number of transfers to give a desired separation and to calculate the purity that will be attained.

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Sabadilla Alkaloids VI.

Separation of Veratridine and Cevadine by Countercurrent Distribution. *p*H *vs.* Partition Coefficients*

By HYMAN MITCHNER† and LLOYD M. PARKS

Contrary to previously reported results of partition studies, the plot of the partition coefficient *vs.* *p*H for both cevadine and veratridine has been shown to be linear. The two alkaloids were separated from each other in a high state of purity by countercurrent distribution with chloroform and 0.5 *M* citrate buffer at *p*H 3.95 with a relatively small number of transfers as well as on a partition column using the same conditions. A much greater number of transfers was required to separate them by countercurrent distribution with benzene and 0.5 *M* phosphate buffer at *p*H 5.85. No effective separation could be made on a partition column using the latter conditions. A nonaqueous titration for the alkaloids in benzene or chloroform solution was perfected and an ultraviolet spectral ratio method for detection and analysis of cevadine occurring with veratridine was applied.

In a previous report (1) on the relationship between *p*H and partition coefficients of cevadine and veratridine the latter appeared to behave in a nonideal fashion in the benzene-buffer system. When the *p*H was plotted against the log of the partition coefficient, instead of obtaining two parallel straight lines of slope +1 for the alkaloids, the veratridine deviated considerably from the expected slope with a resultant intersection of the two plots. This was ascribed to a possible association of one of the alkaloids with the solvent. Since there exists considerable interest in the separation of cevadine and veratridine, it was decided to study this problem further. Utilizing countercurrent distribution it was possible to separate cevadine and veratridine in a high degree of purity. Improvements in analytical techniques resulted in more accurate data, especially in lowering titration errors. The reported discrepancy was resolved and veratridine was found to behave in an ideal fashion.

The earlier report (1) showed that cevadine and veratridine could be separated by partition chromatography using citrate buffer as the stationary phase and chloroform as the mobile phase. In the present report partition studies were made in benzene-phosphate buffer and in chloroform-citrate buffer systems. From the results it was evident that the β values[1] obtained in the latter system were significantly greater than the β values of the benzene-phosphate system.

A comparison was made of (*a*) the effectiveness of benzene-phosphate buffer and chloroformcitrate buffer systems on partition columns and (*b*) the effectiveness of the two systems in countercurrent distributions. It was apparent from the partition studies that the chloroform-citrate system should yield pure cevadine and veratridine in a fraction of the number of transfers used in the benzene-phosphate system. This was borne out in the results of the countercurrent work.

Beroza (2) has utilized the elution pattern of chromatographic zones, as determined by the ultraviolet absorbance, as an index of purity. He suggested that if the spectrum of the material had a maximum and a minimum, the wavelengths corresponding to those points would be the best wavelengths to measure the absorbances. If absorbance ratios remained constant for a zone, it constituted additional evidence of purity.

An adaptation of this method can be applied to mixtures containing only cevadine and veratridine. In the ultraviolet, cevadine and veratridine have spectra as shown in Fig. 1.[2] Previously, the extinction coefficient of veratridine at 262 mμ had been used as a criterion of purity. We have shown that all three peaks of veratridine and the single peak of cevadine obey Beer's law. Since cevadine has very little absorbance at peak *C* of veratridine (293 mμ) and absorbs quite strongly at 217 mμ, which is almost the same wavelength at which peak *A* of veratridine absorbs quite strongly, this suggests the use of the ratio of peaks *A*:*B* or *A*:*C* as a criterion of purity. The presence of cevadine would affect peak *C* of the veratridine very

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† WARF Research Assistant, 1954–56.
[1] β value is defined as the ratio of the partition coefficients such that β is always greater than 1.
[2] All spectral measurements were carried out on a Cary model 11MS recording spectrophotometer.

little. It would increase peak *B* somewhat, but would have a marked effect on peak *A*. Thus a small increase in the *B:C* ratio over the ratio for pure veratridine, coupled with a more marked increase in the *A:C* ratio, would be good evidence of cevadine as an impurity.

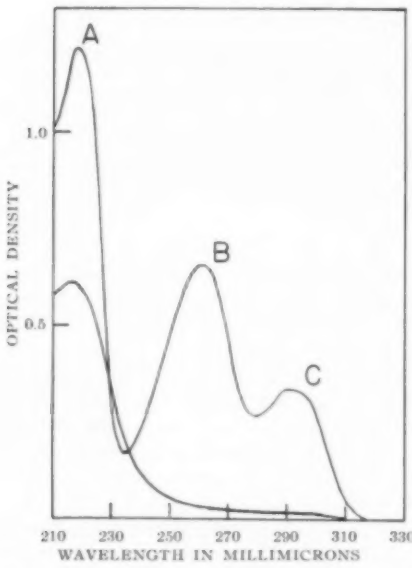

Fig. 1—Ultraviolet spectra of cevadine and veratridine; the former with a single peak, the latter with three peaks, *A*, *B* and *C*.

Not only does the ratio of *A:C* show the presence of a cevadine impurity but it also can be used to show the amount of cevadine present. Because cevadine has some ultraviolet absorbance at 293 mμ, the amount is not exact unless a correction for this absorbance is made. However, even neglecting the slight absorbance of cevadine at 293 mμ, a good relative value can be obtained. At 218 mμ, veratridine has an extinction coefficient, *E*, of 3260 (*E* is taken as one hundred times the extinction coefficient with the concentration in grams per liter). Cevadine at 218 mμ has an equivalent extinction coefficient of 1600, so that any increase at *A* over the theoretical for veratridine can be attributed to a concentration of cevadine approximately twice that necessary for the same absorbance at *A* for veratridine. For a sample of veratridine isolated and purified by countercurrent distribution, the following ratios were obtained: $A:B = 1.80 \pm 0.02$; $A:C = 3.50 \pm 0.03$; $B:C = 1.96 \pm 0.02$.

EXPERIMENTAL

Extraction of Alkaloids.—Twenty kilograms of 20-mesh Sabadilla seed obtained from S. B. Penick and Company was extracted by a modified cold percolation technique. The percolator was set up to allow a continuous flow of solvent into the top and out of the bottom with constant stirring of the contents. The rate of flow was 2.5 ml. per minute. Petroleum ether (Skelly B) was used as the first solvent and extraction was continued until most of the fats were removed, as shown by the evaporation of a 100-ml. sample of percolate. A total of 240 L. of Skelly B was used. The residue was air-dried and extracted with ammonia-saturated-chloroform in an identical way until the percolate gave only a slight alkaloidal test with Mayer's reagent. A total of 200 L. of chloroform percolate was collected. The chloroform was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*, yielding 520 Gm. of a brown amorphous alkaloidal mixture.

Concentration of Cevadine and Veratridine.—A 50-Gm. portion of the chloroform extract was shaken with 800 ml. of benzene for four hours. The insoluble residue was filtered under suction, the benzene solution evaporated to dryness under reduced pressure, and the residue taken up in 400 ml. of chloroform. The chloroform solution was first extracted with two 200-ml. portions of 0.5 *M*, *p*H 4.4 citrate buffer and then with 200-ml. portions of 10% sulfuric acid until the acid washings were negative to Mayer's reagent. The total sulfuric acid extract (4 L.) was chilled and made strongly alkaline with concentrated ammonium hydroxide, keeping the solution cold in an ice bath. The turbid mixture was extracted with two 2-L. portions of chloroform, the chloroform extract dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 20 Gm. of alkaloidal mixture composed primarily of cevadine and veratridine.

Analysis of Countercurrent Distributions.—The organic phase used in the countercurrent distributions was benzene or chloroform. The aqueous phase was 0.5 *M* phosphate buffer or 0.5 *M* citrate buffer. Each tube of the Craig countercurrent apparatus[1] when withdrawn contained 10 ml. of buffer and 10 ml. of organic phase with the alkaloid distributed between the two phases. To facilitate the preparation of the distribution curve it was found convenient to drive essentially all the alkaloid into the organic phase by the addition of excess alkali. For 10 ml. of 0.5 *M* phosphate buffer of *p*H 5 or greater, it was found that 1 ml. of 5 *N* aqueous sodium hydroxide was sufficient to produce a strongly alkaline aqueous phase. For 10 ml. of 0.5 *M* citrate buffer of *p*H 3 or greater, it was found that 2 ml. of 5 *N* aqueous sodium hydroxide produced a sufficiently alkaline aqueous phase.

In the countercurrent distributions using benzene as the organic phase the following procedure was used: To each fourth or fifth tube was added 1 ml. of 5 *N* sodium hydroxide, the two phases thoroughly shaken, 1 ml. of the supernatant benzene layer withdrawn in a pipet and the alkaloidal content determined by nonaqueous titration in a 5-ml. beaker

[1] The Craig apparatus used was a 200-plate robot driven instrument manufactured by H. O. Post, Scientific Instrument Co., Maspeth, N. Y.

using a magnetic stirrer. The alkaloid was titrated with 0.01 *N* perchloric acid dissolved in glacial acetic acid utilizing a 2-ml. microburet calibrated in 0.01-ml. divisions. As indicator, 2 drops of 0.02% quinaldine red in glacial acetic acid, benzene, or chloroform, was found to work well. The color change was sharp, going from red to colorless. The first appearance of the colorless solution was taken as the end point. There was a gradual reappearance of the red color when the solution was exposed to air.

Titration of the chloroform solutions required some modification. It was found that the blank value using only chloroform and indicator was considerably higher than for benzene. Furthermore, the end point was not nearly as sharp as with the benzene solutions. The addition of an equal volume of Skelly C to the chloroform solution was found to reduce the blank value and sharpen the end point.

During titration of some of the more concentrated alkaloid solutions in benzene or in Skelly C-chloroform, precipitation of the perchlorate salt sometimes occurred. This precipitate was white and, if the solution was stirred well, did not interfere with the sharpness of the end point.

Analysis of Partition Equilibria.—The partition equilibria were analyzed analogously to the countercurrent distributions. No alkali was added. The benzene or chloroform layers were separated after equilibration and 2-ml. aliquots of the organic phase withdrawn by pipet, taking care to avoid any contamination from the aqueous phase. The organic solutions were then titrated in 5-ml. or 10-ml. beakers. The concentration of alkaloid in the stock solutions of the alkaloids in benzene or chloroform was determined by titration of 2-ml. portions of the stock solutions. The partition coefficient, K, was taken as $K = [B]$ organic/($[B]$ stock $- [B]$ organic).

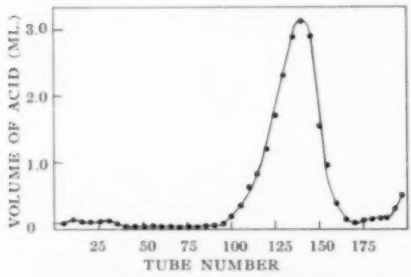

Fig. 2.—Countercurrent distribution of 9 Gm. of cevadine-veratridine mixture between benzene and *p*H 6.0 phosphate buffer.

Separation of Cevadine and Veratridine.—The first countercurrent run was made on 9 Gm. of the cevadine-veratridine concentrate prepared as described and dissolved in 270 ml. of benzene. Each tube of the apparatus was filled with 10 ml. of 0.5 *M*, *p*H 6.0 phosphate buffer and the benzene solution of the alkaloid introduced in the first 27 tubes. Figure 2 shows the distribution pattern after 190 transfers. Tubes 91–140 and 141–170 were collected as two units. To the former was added 50 ml. of 5 *N* sodium hydroxide and to the latter 30 ml. of the alkali. After shaking thoroughly, the benzene layers were removed, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo*.

Two grams of the alkaloidal mixture obtained from tubes 91–140 was dissolved in 40 ml. of benzene, and, under the same conditions as the previous distribution, placed in the first 4 tubes of the apparatus. Distribution was carried out for 223 transfers with the results shown in Fig. 3. Tubes 100–135 were

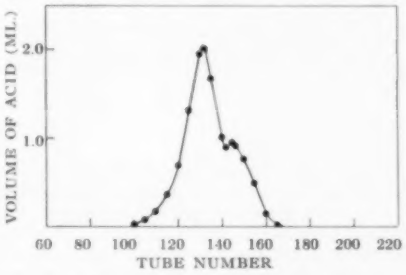

Fig. 3.—Countercurrent distribution of material in tubes 91–140 of Fig. 2 between benzene and *p*H 6.0 phosphate buffer.

combined, 35 ml. of 5 *N* sodium hydroxide added and the two phases shaken thoroughly in a separatory funnel. The benzene layer was separated and the alkaloidal content worked up as before. The amorphous alkaloid was taken up in a minimum amount of hot absolute ethanol. On standing, a crystalline product formed which was filtered off and recrystallized from ethanol. These crystals were dried under high vacuum at 120° for 24 hours, m. p. 212°. Yield 1.3 Gm. The ultraviolet and infrared spectra were identical with those of cevadine. A countercurrent distribution with 50 mg. of this product and 50 mg. of an authentic sample of cevadine dissolved in 10 ml. of benzene and distributed on

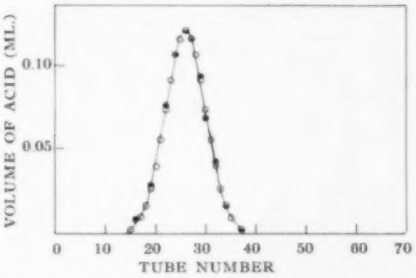

Fig. 4.—Countercurrent distribution of cevadine between benzene and *p*H 6.65 phosphate buffer. ● = experimental; ○ = theoretical.

the apparatus for 70 transfers with *p*H 6.65, 0.5 *M* phosphate buffer as the lower phase and benzene as the upper phase gave only a single peak. Both experimental and theoretical values coincided (see Fig. 4). Tubes 136–170 of the second distribution

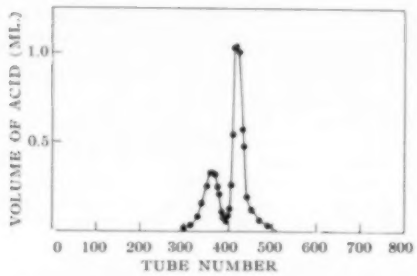

Fig. 5.—Countercurrent distribution of material in tubes 136–170 of Fig. 3 and tubes 141–170 of Fig. 2 between benzene and *p*H 5.8 phosphate buffer.

were combined, the alkaloidal content isolated as described and added to the material obtained from tubes 141–170 of the first run. Three grams of this combined material was dissolved in 50 ml. of benzene and placed in the first five tubes of the apparatus. Phosphate buffer of *p*H 5.8 (0.5 *M*) was used as the lower phase, benzene as the upper phase. After 195 transfers, tubes 0–19 and 180–199 inclusive were withdrawn and replaced with fresh buffer and benzene. The apparatus was then adjusted for recycling and distribution continued for a total of 780 transfers. Each fifth tube was withdrawn and its contents analyzed. Figure 5 shows the distribution pattern

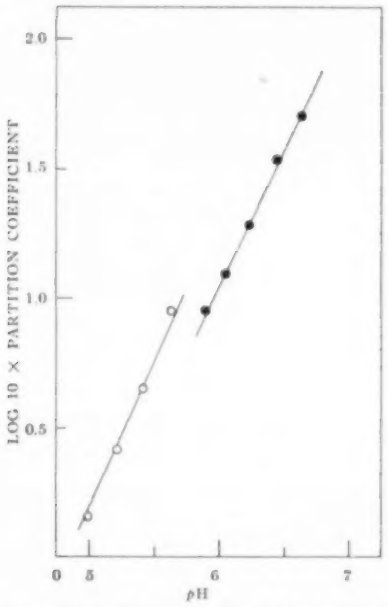

Fig. 6.—Log of partition coefficient *vs.* *p*H for cevadine between benzene–0.5 *M* citrate ○ and benzene–0.5 *M* phosphate buffers ●.

obtained. Tubes 300–390 were combined and processed as described to yield 500 mg. of cevadine. Tubes 400–475 were combined and worked up to yield 1.4 Gm. of amorphous alkaloid whose ultraviolet and infrared spectra agreed with those for veratridine.

Partition Studies.—Sufficient cevadine and veratridine were dissolved in benzene and in chloroform to make stock solutions containing about 1 mg./ml. Equal volumes (10 ml.) of stock solution and buffer were measured with pipets and equilibration was carried out in stoppered 18 × 150-mm. Pyrex culture tubes which were shaken for 40 minutes on an electric shaker.

The buffer solutions employed were either 0.5 *M* citrate or 0.5 *M* phosphate. They were made by mixing solutions of mono- and disodium phosphate or solutions of citric acid and sodium citrate. The *p*H values were measured on a Beckman model G *p*H meter. It was noted that in the overlap region of *p*H values in the two buffer systems, the same *p*H gave higher values for the partition coefficient with the citrate buffers. Figure 6 illustrates this phenomenon on the partitioning of cevadine between benzene and citrate or phosphate buffer systems. Since the partitioning effect was not wholly dependent on the hydrogen ion concentration, it was necessary to use all citrate or all phosphate buffers for each run. Results of the determination of the partition coefficients are shown in Tables I and II. Plots of log (10 × partition coefficient) *vs.* *p*H are shown in Figs. 7 and 8.

TABLE I.—PARTITION COEFFICIENTS OF CEVADINE AND VERATRIDINE IN BENZENE USING PHOSPHATE BUFFERS

	Partition Coefficient	
*p*H	Cevadine	Veratridine
4.93	...	0.160
5.16	0.195	0.266
5.38	0.264	0.411
5.60	0.498	0.751
5.88	0.896	1.490
6.04	1.190	2.086
6.23	1.840	3.605
6.42	3.540	5.950
6.62	6.790	12.19

TABLE II.—PARTITION COEFFICIENTS OF CEVADINE AND VERATRIDINE IN CHLOROFORM USING CITRATE BUFFERS

	Partition Coefficient	
*p*H	Cevadine	Veratridine
3.07	...	0.222
3.27	0.066	0.321
3.47	0.111	0.523
3.67	0.212	1.022
3.87	0.348	1.816
4.17	0.722	4.111
4.38	1.325	7.91
4.60	2.41	14.33
4.78	4.907	...
4.98	9.34	...

Countercurrent Separations Utilizing the Partition Data.—From Figs. 7 and 8 it can be seen that the *p*H for the optimum separation of cevadine and veratridine with chloroform and citrate buffer

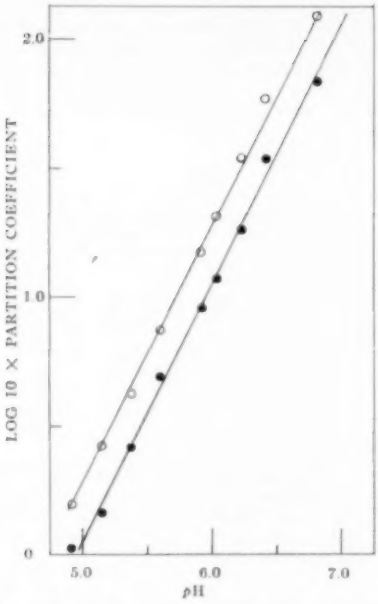

Fig. 7.—Log of partition coefficient *vs.* *p*H for veratridine ○ and cevadine ● between benzene and 0.5 *M* phosphate buffer.

should be 3.95 whereas with benzene and phosphate buffer the optimum *p*H should be 5.84. The optimum conditions of $K_1 = {}^1/K_2$ can be readily determined from the log $10K$ *vs.* *p*H plots by determining that *p*H at which the parallel lines are equidistant from log $10K = 1$ (3). Under these optimum conditions the relationship $n = 2.3 \log R/C$

where $R = \frac{T_{1\ \max.}}{T_1} = \frac{T_{2\ \max.}}{T_2} = \frac{T_{1\ \max.} + T_{2\ \max.}}{T_{n/2}}$, $\quad C = \frac{(K-1)^2}{8K}$

and n is the number of transfers (3), can be applied to show the number of transfers to obtain the R ratios as shown in Table III.

TABLE III.—NUMBER OF TRANSFERS TO OBTAIN PRESET PARTITION RATIOS WITH CEVADINE AND VERATRIDINE UNDER OPTIMUM SEPARATION CONDITIONS

Ratio (R)	Number of transfers: Cevadine and Veratridine *p*H 5.84 (Phosphate buffer-benzene system)	Number of transfers: Cevadine and Veratridine *p*H 3.95 (Citrate buffer-chloroform system)
5:1	210	19
10:1	300	27
15:1	353	32
20:1	390	35

Two hundred milligrams of the cevadine-veratridine concentrate prepared as described was dissolved in 10 ml. of chloroform and introduced into the first tube of the Craig apparatus. The lower phase was chloroform, the upper phase was 0.5 *M*, *p*H 3.95 citrate buffer. Twenty-seven transfers were run and the analysis carried out by nonaqueous titration on 2-ml. aliquots of the organic phase after alkali treatment. Figure 9 shows the distribution obtained. R by theory, 10:1; found, 10.3:1.

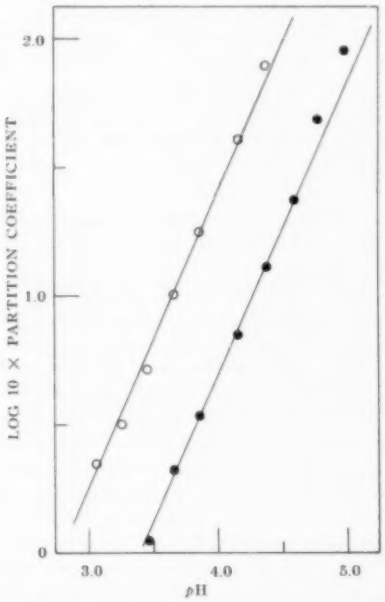

Fig. 8.—Log of partition coefficient *vs.* *p*H for veratridine ○ and cevadine ● between chloroform and 0.5 *M* citrate buffer.

The above distribution was repeated, but for 35 transfers. Figure 10 shows the distribution obtained. R by theory, 15:1; found, 14.7:1.

The apparatus was filled with 0.5 *M*, *p*H 5.85 phosphate buffer as the lower phase. A 200-mg. portion of the alkaloidal concentrate of cevadine and veratridine was dissolved in 10 ml. of benzene and placed in the first tube. Benzene was run as the upper

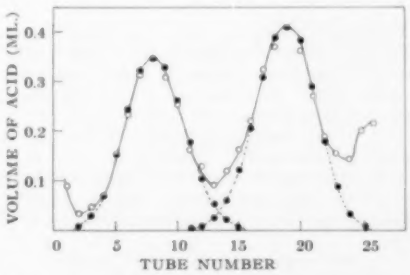

Fig. 9.—Twenty-seven transfer countercurrent distribution of a cevadine-veratridine mixture between chloroform and *p*H 3.95 citrate buffer. ○ = experimental; ● = theoretical.

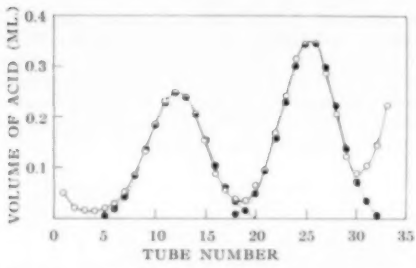

Fig. 10.—Thirty-five transfer countercurrent distribution of a cevadine-veratridine mixture between chloroform and *p*H 3.95 citrate buffer. ○ = experimental; ● = theoretical.

phase for 100 transfers. Figure 11 shows the distribution obtained. *R* by theory, 2.14:1; found, 2.14:1.

A second run was made with the phosphate buffer-benzene system with an altered alkaloidal sample that contained a greater amount of cevadine than those samples used in the previous distributions. The conditions were kept the same and 96 transfers made. Figure 12 shows the distribution obtained. *R* by theory, 2.09:1; found, 2.10:1.

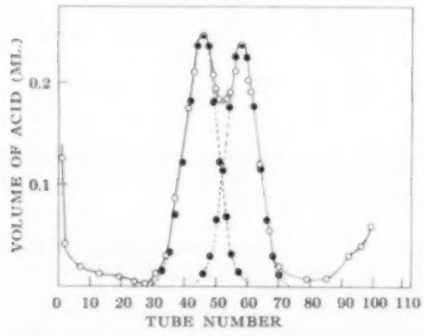

Fig. 11.—One hundred transfer countercurrent distribution of a cevadine-veratridine mixture (1:1) between benzene and *p*H 5.85 phosphate buffer. ○ = experimental; ● = theoretical.

Partition Chromatography.—Partition columns were prepared with 15 Gm. of silicic acid (Mallinckrodt 100 mesh) and 15 ml. of buffer and packed in tubes of 1.2 cm. diameter (1, 4). The columns were packed to allow a flow rate of about 1 ml. per minute. Two hundred-milligram samples of the alkaloidal mixture were dissolved in 1 ml. of the organic phase used to elute the column. Constant volume fractions were collected and the alkaloidal content determined by nonaqueous titration of aliquots.

Runs were made using (*a*) 0.5 *M* phosphate buffer, *p*H 5.90 as the internal phase, benzene as the external phase, and (*b*) 0.5 *M* citrate buffer, *p*H 3.95 as the internal phase, chloroform as the external phase. In (*a*) 18-ml. samples were collected and 2-ml. aliquots titrated. After 108 fractions, 300 ml. of chloroform was run through the column, collected in 25-ml. fractions and 2-ml. aliquots titrated. Figure 13 shows the results obtained. Ultraviolet spectra were taken on selected tubes and also analyzed by the method of ratios. Table IV shows the results obtained.

TABLE IV.—ULTRAVIOLET SPECTRAL RATIOS FROM PARTITION CHROMATOGRAM (*a*)

Tube	Ratio *A*:*B*	Ratio *A*:*C*	Ratio *B*:*C*
Benzene			
36	2.65	4.88	1.84
50	2.54	4.92	1.91
60	2.50	4.77	1.91
Chloroform			
1	3.13	6.24	1.99
4	3.17	5.96	1.88

In run (*b*) 5.6-ml. samples were collected and 1-ml. aliquots titrated. Forty tubes were collected. Figure 14 shows the separation obtained. Ultraviolet spectra were also taken and analyzed. Table V shows the results obtained.

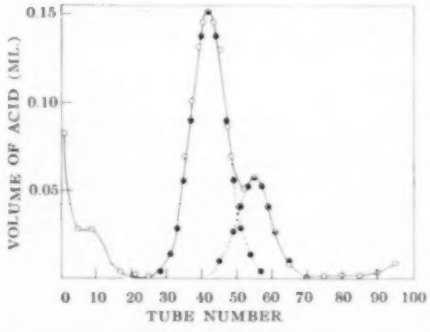

Fig. 12.—Ninety-six transfer countercurrent distribution of a cevadine-veratridine mixture (3:1) between benzene and *p*H 5.85 phosphate buffer. ○ = experimental; ● = theoretical.

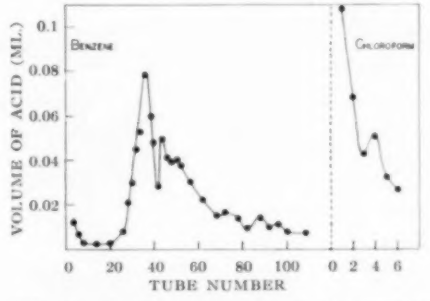

Fig. 13.—Partition chromatogram of a cevadine-veratridine mixture on silicic acid with *p*H 5.90 phosphate buffer as internal phase.

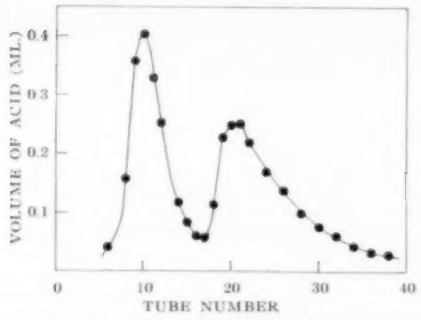

Fig. 14.—Partition chromatogram of a cevadine-veratridine mixture on silicic acid with *p*H 3.95 citrate buffer as internal phase and chloroform as external phase.

TABLE V.—ULTRAVIOLET SPECTRAL RATIOS OF PARTITION CHROMATOGRAM (*b*)

Tube	Ratio *A*:*B*	Ratio *A*:*C*	Ratio *B*:*C*
8	1.8	3.48	1.95
11	1.79	3.49	1.95
13	1.79	3.48	1.95
18	3.82	7.66	1.95
22	No veratridine peaks		
26	"		
30	"		

DISCUSSION AND SUMMARY

1. Both cevadine and veratridine behave ideally in partition studies in that the plot of the log of the partition coefficient *vs.* *p*H is linear. Their slopes are very close to +1 and this property can be utilized in identifying the alkaloids at various *p*H's in countercurrent work. It can also be used to determine the optimum *p*H for the separation of the two alkaloids. The reported discrepancy (1) involving the nonideal behavior of veratridine has been resolved. It was probably due to samples of insufficient purity or to the analytical procedures used.

2. Countercurrent distribution utilizing chloroform and *p*H 3.95, 0.5 *M* citrate buffer can be used to separate cevadine and veratridine in a high state of purity in a relatively small number of transfers although the system of phosphate buffer and benzene will separate the two alkaloids when a much larger number of transfers is used.

3. An ultraviolet spectral method of analysis for cevadine occurring along with veratridine has been applied.

4. A method of titrating cevadine and veratridine in benzene or chloroform solutions has been developed. The method should be applicable to other alkaloids of comparable basicity.

5. Partition chromatography studies using optimum or near optimum conditions as found by partition studies have indicated that effects other than partitioning may be occurring during column separations. Benzene-phosphate buffer was found to yield only a mixture of cevadine and veratridine from a silicic acid column. Furthermore, all the cevadine and veratridine could not be eluted from the column with benzene. With chloroform the alkaloids were eluted. Ultraviolet spectral analysis indicated that the material eluted with benzene was rich in veratridine whereas the material remaining on the column was rich in cevadine. This "sticking" of the cevadine is also seen with chloroform-citrate buffer columns as a "tailing" effect. The veratridine as separated by the chloroform-citrate buffer systems was shown to be quite pure when coming off the silicic acid column. The slight carryover to the cevadine could result from the method used to add the material to the column as the veratridine appears to "tail" very little under optimum conditions.

Since the "tailing" effect is characteristic of adsorption columns it is believed that adsorption as well as partitioning occurs on the column. This is not unreasonable since the column is silicic acid and the substances placed on the column are basic. The net effect appears to be that if the alkaloids are eluted from the column under partition conditions such that there is a fairly large difference in partition coefficients and conditions near those for optimum separation, separation can be obtained in spite of the "tailing." However, if the partition coefficients are too close together or conditions are too far from optimum, the sharpness of the separation is decreased. In some systems, although separation is possible by countercurrent distribution, it may not be possible to effect a separation on a partition column.

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The Nonaqueous Titration of PAS, Sodium PAS, and Some Commercial Preparations*

By L. G. CHATTEN

A new nonaqueous method is presented which permits the visual titration of *p*-aminosalicylic acid and its sodium salt. Acetone is the solvent employed for the free acid, whereas anhydrous methanol is used to dissolve the sodium salt. The indicator, in both instances, is thymol blue. The investigation has been extended to some commercial tablet preparations.

PARA-AMINOSALICYLIC ACID and its salts, which have gained popularity in recent years as tuberculostatics, have been assayed by a variety of methods. Oberweger, *et al.* (1), and Coppini (2) employed bromination, while Tarnoky and Brews (3) as well as Pesez (4, 5) used diazotization, which is the basis of the U. S. P. XV method (6). Ultraviolet spectrophotometry is the prescribed method of N. N. R. (7), while Matta and Nunes (8) employed a modified Volhard method and Roy (9) analyzed the compound by decarboxylation.

Rao, *et al.* (10), compared the bromination, diazotization, spectrophotometric, and decarboxylation methods and concluded that the most satisfactory procedure, as far as they were concerned, was to perform a decarboxylation and follow this by diazotization. Using this technique, unfortunately, requires five hours to perform a single complete determination. Considerable variation was found with the diazotization procedure alone. Butler and Ramsey (11), in their paper on the nonaqueous titration of this drug and its sodium salt, pointed out the disadvantage of the diazotization method which must rely on an outside indicator. Experience in this laboratory with the U. S. P. technique bears out the findings of the aforementioned authors.

The potentiometric method of Butler and Ramsey appears to work for pure crystalline material. It was noted, however, that these workers did not indicate whether their technique was applicable to commercial tablets. When an attempt was made, in this laboratory, to apply their method, no end point could be detected because of interference by tablet excipients.

Gautier and Pellerin (12) claim to have successfully titrated sodium *p*-aminosalicylate in a medium of glacial acetic acid, using methyl violet or tropeoline 00 as the indicator. Butler and Ramsey reported that a potentiometric titration in glacial acetic acid gave a curve with a poorly defined end point, and it was found in this laboratory that a visual titration as described by Gautier and Pellerin gave no detectable end point. Das and Palit (13) utilized an equal mixture of ethylene glycol and isopropanol to titrate sodium *p*-aminosalicylate potentiometrically. The authors admitted, that, while the titration can be followed with thymol blue, the color change is not sufficiently sharp. Stockton and Zuckerman (14), in a simultaneous publication, employed the same solvent system as Das and Palit for the potentiometric titration of the salt.

The purpose of this investigation is to provide nonaqueous procedures for both the free acid and the salt as well as the commercial products, which can be readily carried out by visual titration.

EXPERIMENTAL

Apparatus.—5-ml. or 10-ml. buret, graduated in 0.01 ml., electromagnetic stirrer, 125-ml. suction flask, and small Büchner funnel.

Reagents.—Acetone, A.C.S. grade; anhydrous methanol, A.C.S. grade; benzoic acid, A.C.S. grade; potassium hydroxide, A.C.S. grade; perchloric acid, A.C.S. grade; dioxane, A.C.S. grade; potassium acid phthalate, A.C.S. grade; potassium hydroxide 0.1 *N* in anhydrous methanol; perchloric acid, 0.05 *N* in dioxane; thymol blue indicator 0.5% in anhydrous methanol.

Standardization of Titrants.—*Potassium hydroxide in methanol.*—Accurately weigh approximately 200 mg. of benzoic acid and dissolve in 50 ml. of chloroform, add 4 drops of thymol blue indicator, and titrate to a lilac color. A blank on the solvent system is approximately 0.10 ml. of titrant. *Perchloric acid in dioxane.*—This is standardized by the conventional method of Seaman and Allen (15) using potassium acid phthalate.

Procedures.—(*a*) To assay crystalline *p*-aminosalicylic acid, accurately weigh a sample of 80–100 mg. into a 150-ml. beaker or flask, dissolve in 50 ml. of acetone by stirring electromagnetically, add 4 drops of thymol blue indicator, cover the beaker, and titrate with 0.1 *N* KOH in methanol to a blue end point. One ml. of 0.1 *N* KOH in methanol is equivalent to 15.31 mg. of PAS.

* Received April 17, 1956, from the Pharmaceutical Chemistry Section, Department of National Health and Welfare, Ottawa, Canada.
Presented to the Scientific Section, A. PH. A., Detroit meeting, April, 1956.

(*b*) For commercial *p*-aminosalicylic acid preparations, weigh and powder 20 tablets. Place an accurately weighed sample equivalent to 80–100 mg. of the drug in a 150-ml. beaker. Add 40 ml. of acetone and stir electromagnetically for ten minutes. Suction filter through Whatman No. 1 paper into a 125-ml. flask. Wash the beaker and funnel with 10 ml. of acetone and filter as before. Add 4 drops of thymol blue solution, protect the flask from the atmosphere and titrate to a blue end point with 0.1 *N* KOH in methanol.

(*c*) To assay crystalline sodium *p*-aminosalicylate, accurately weigh a sample of 40–50 mg. into a 150-ml. beaker or flask, dissolve in 50 ml. of anhydrous methanol by stirring electromagnetically, add 4 drops of thymol blue, and titrate with 0.05 *N* perchloric acid in dioxane to a peach end point. One ml. of 0.05 *N* perchloric acid in dioxane is equivalent to 10.56 mg. of sodium PAS.

(*d*) For commercial sodium *p*-aminosalicylate preparations, weigh and powder 20 tablets. Place an accurately weighed sample equivalent to about 50 mg. of the drug in a 150-ml. beaker. Add 40 ml. of anhydrous methanol and stir electromagnetically for ten minutes. Suction filter through Whatman No. 1 paper into a 125-ml. flask. Wash the beaker and funnel with 10 ml. of methanol and filter as before. Add 4 drops of thymol blue solution and titrate to a peach end point with 0.05 *N* perchloric acid in dioxane.

RESULTS AND DISCUSSION

Para-aminosalicylic acid is readily soluble in acetone and the end point with thymol blue, when the compound is titrated with potassium hydroxide in methanol, is very sharp. The visual end point was checked potentiometrically on a Fisher titrator which was equipped with the glass-calomel electrode system. Figure 1 illustrates that the two end points coincide. To test the precision of the method for this drug, five consecutive determinations were run on a U. S. P. quality sample of crystalline material and the standard deviation calculated. The results are recorded in Table I. Then several samples of commercial tablets were analyzed by the proposed procedure and comparative assays were performed by the U. S. P. XV nitrite method. The results of this study appear in Table II. The nonaqueous method is rapid and accurate and generally better agreement occurs between replicates with it than with the nitrite procedure.

Table I.—Titration of a Single Lot of Crystalline *p*-Aminosalicylic Acid in Acetone

Taken, mg.	Recovered, mg.	Recovery, %
76.0	75.4	99.2
82.1	81.5	99.3
88.6	87.6	98.9
91.4	90.7	99.2
91.3	90.2	98.8
	Mean	99.1 ± 0.2

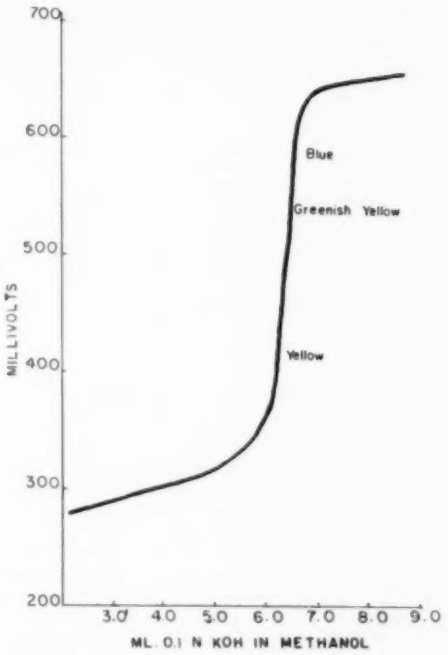

Fig. 1.—Titration of PAS in acetone.

Table II.—Comparative Assays of *p*-Aminosalicylic Acid Tablets

Product	Nonaqueous	Nitrite Method
A	98.8	99.3
	98.4	101.8
	99.1	
B	101.7	100.2
	101.1	99.8
	101.1	
C	101.1	103.8
	101.3	102.8
	100.4	
D	99.4	101.2
	99.4	102.3
	99.6	
E	102.6	104.0
	102.0	103.2
	102.4	

Potassium hydroxide in methanol has been used as the titrant in this laboratory for some time, and is much simpler to prepare than the conventional methoxides which require metallic sodium, potassium, or lithium. Recent publications from this laboratory (16, 17) have shown it to be equally satisfactory for the determination of the bile acids and the barbituric acids. Indeed, Caldin and Long (18) proved that in reality a solution of NaOH in methanol did not differ in methoxide content from one prepared using metallic sodium.

Sodium *p*-aminosalicylate is readily soluble in anhydrous methanol. When the compound is titrated in this solvent with perchloric acid in di-

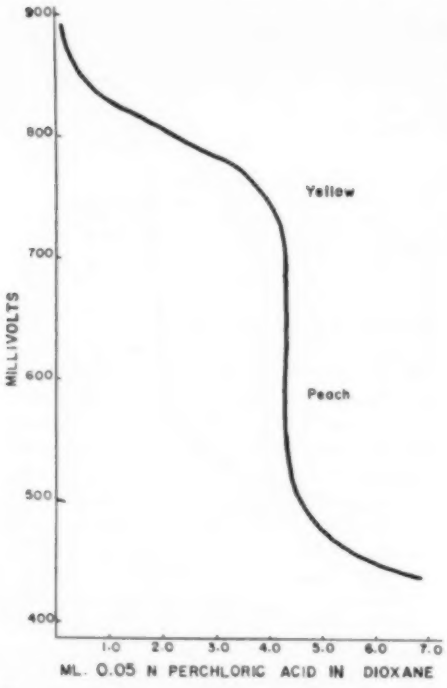

Fig. 2.—Titration of sodium PAS in methanol-solvent system.

oxane, the end point with thymol blue is readily reproducible with a little practice. Figure 2 illustrates that the visual end point coincided with the one determined potentiometrically when a Fisher titrator equipped with a glass-calomel electrode combination was employed. To test the precision of the method for this drug, five consecutive determinations were performed on a sample of U. S. P. crystalline material and the standard deviation calculated. The results are given in Table III.

TABLE III.—TITRATION OF A SINGLE LOT OF CRYSTALLINE SODIUM *p*-AMINOSALICYLATE IN METHANOL

Taken, mg.	Recovered, mg.	Recovery, %
51.1	51.0	99.8
48.2	48.2	100.0
51.7	51.5	99.6
49.8	49.8	100.0
50.4	50.4	100.0
	Mean	99.8 ± 0.23

It seemed logical, then, to apply this technique to commercial products. The results of this study appear in Table IV together with comparative assays which were determined by the U. S. P. nitrite method. The nonaqueous method is rapid and accurate and the agreement between replicates appears to be satisfactory. Such a procedure would seem to be preferable to the nitrite method which employs an outside indicator.

TABLE IV.—COMPARATIVE ASSAYS OF SODIUM *p*-AMINOSALICYLATE TABLETS

Product	Nonaqueous	Nitrite Method
A	93.8	96.7
	93.3	96.3
	93.4	
B	105.6	108.1
	106.5	107.4
	105.9	
C	99.6	98.1
	99.4	98.5
	99.4	
D	103.0	99.2
	102.2	100.0
	102.6	
E	88.0	88.1
	88.6	89.0
	88.5	

SUMMARY

1. New nonaqueous techniques have been devised for the assay of *p*-aminosalicylic acid and its sodium salt, which are rapid, accurate, and can be performed visually.

2. The procedures have been successfully applied to market samples of commercial tablet preparations.

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Pilocereine, a Cactus Alkaloid*

By C. E. POWELL and K. K. CHEN

Pilocereine hydrochloride, salt of pilocereine, a cactus alkaloid isolated from *Lophocereus Schottii* and *Pachycereus marginatus*, was studied pharmacologically. Its action by vein was determined on the blood pressure, respiration, and small intestine of anesthetized dogs and cats. The blood pressure action of the alkaloid was also studied in anesthetized roosters and rats. Pilocereine was found to lower the blood pressure of dogs and roosters while the rat's blood pressure was elevated. In anesthetized cats small doses first lowered and then raised blood pressure. Larger doses only lowered the blood pressure. Pilocereine relieves methacholine- and histamine-induced spasm of the isolated small intestine of rabbits and guinea pigs. The alkaloid also seems to have an antimalarial action when fed to canaries.

THE DISCOVERY of an alkaloid, anhalonine, from "mescal buttons" by Lewin (1–3) in 1888 aroused much interest in the chemical composition of the *Cactaceae* family (4). From *Lophocereus Schottii* Heyl (5) isolated an amorphous alkaloid, pilocereine, which Djerassi (6) recently obtained in crystalline form from the same species growing in the state of Sonora in northern Mexico. This crystalline alkaloid was found to have the empirical formula $C_{20}H_{42}N_2O_4$. Later Djerassi (7) isolated the same alkaloid from *Pachycereus marginatus*, *L. australis* and *L. gatesii*.

Pharmacological studies by Heyl (5) showed that pilocereine hydrochloride induced paralysis in the frog. It reduced contractibility of the ventricle without changing the heart rate. The paralyzing action of the alkaloid was not demonstrated in warm-blooded animals, which died with signs of sudden heart stoppage. The pharmacological action of cereine, another alkaloid from *P. marginatus*, has been reported by de Lille (8). In sublethal doses this alkaloid elicited mydriasis, tachycardia, clonic convulsions, and depressor response. It had no effect on skeletal muscle and only slightly inhibited smooth muscles. The recent investigation by Djerassi (7) showed that cereine and pilocereine had a similar chemical structure.

Because of the recent interest in the cactus alkaloids we undertook a further investigation of the pharmacological properties of pilocereine.

EXPERIMENTAL

Methods and Materials.—Pilocereine, isolated from *L. Schottii* and *P. marginatus*, was kindly supplied by Dr. Carl Djerassi, Wayne University, Detroit, Mich., in the form of hydrochloride. The salt was easily soluble as a 1% solution in distilled water.

The pharmacological action of pilocereine was determined on the blood pressure, respiration, and small intestine of nine dogs and nineteen cats. In addition, the blood pressure effects of the alkaloid were recorded from three adult roosters and two female albino rats. All animals used in the blood pressure experiments were anesthetized intraperitoneally with sodium phenobarbital (170 to 190 mg./Kg.). Doses of the alkaloid ranging from 0.25 to 4 mg./Kg. were given through the femoral vein. The blood pressure responses were recorded from a cannulated carotid artery via a mercury manometer. One dog and one cat each were given 10 mg./Kg. of the alkaloid by stomach tube and the blood pressure was recorded. Respiratory movements were recorded from a tambour connected to a tracheal cannula. Responses of small intestines were registered through a pulley system attached to a gravity writing lever. The blood pressure effects of pilocereine in rats injected by femoral vein were recorded from a cannulated carotid artery through a small animal manometer (9). The rats were anesthetized intraperitoneally with urethane (1100 to 1250 mg./Kg.). The interaction of pilocereine with epinephrine, ergotoxine ethanesulfonate, and atropine was also investigated. Further, the ganglionic blocking action of the alkaloid was determined in cats by the method of Chen *et al.* (10).

The roosters were anesthetized with sodium phenobarbital (180 mg./Kg.) by vein and the blood pressure responses recorded from the ischiatic artery through a mercury manometer similar to the U. S. P. XIV method (11) for the oxytocic assay of posterior pituitary extracts. Injections of the alkaloid were made through the crural vein.

Four uterine strips and six small intestinal strips from three guinea pigs, and five uterine strips and eight intestinal strips from five rabbits were employed to represent smooth muscle organs. Each preparation was suspended in a 100-ml. muscle chamber containing Tyrode's solution maintained at a temperature of 37.5°. The muscle responses were recorded by a writing lever on a smoked kymograph. Dilutions of pilocereine ranged from 1:100,000 to 1:5,000. The action of the alkaloid was also determined in the perfused frog heart by the method of Howell and Cooke (12). Six large *Rana pipiens* frogs, weighing 80 to 95 Gm., were used with dilutions of pilocereine 1:100,000 to 1:7,500.

* Received February 15, 1956, from the Lilly Research Laboratories, Indianapolis, Ind.

The authors wish to acknowledge the valuable assistance of Messrs. Charles L. Rose, Lester LeCompte, Jr., and James Waddell.

The antimalarial "Q" value (13, 14) of the alkaloid was estimated in canaries infected with the parasite *Plasmodium relictum* by oral and intravenous routes. Toxicity studies of pilocereine were made in starved albino mice, weighing 16 to 21 Gm., by intravenous injection into a tail vein. The mice were observed for twenty-four hours following the injection. The median lethal dose (LD_{50}) was calculated by the Miller and Tainter method (15).

RESULTS

Action in Anesthetized Animals and Roosters.—In dogs all initial doses ranging from 0.25 mg./Kg. to 2 mg./Kg. by vein lowered the blood pressure for periods up to ninety minutes. Occasionally, if the dose was repeated in less than thirty minutes, the blood pressure response was not as great as to the first dose. Likewise, larger doses were less active when repeated. In one dog a 2 mg./Kg. dose of pilocereine lowered the blood pressure 70 mm. Hg for more than ninety minutes. Doses of 1 mg./Kg. in three dogs lowered the blood pressure an average of 62 mm. Hg for one hour. A depressor action also occurred in four dogs, each receiving either 0.5 mg./Kg. or 0.25 mg./Kg. by vein. However, the maximum average depressor response was only 40 mm. Hg and the duration of action fifty minutes. One dog that received 10 mg./Kg. by stomach tube, showed no blood pressure change from normal at the end of one hour. In all experiments the heart rate was reduced twenty to twenty-four beats per minute. Following a brief decrease of amplitude, the respiratory rate increased eight per minute. The amplitude and rate of contraction of the small intestine of dogs were increased following a brief decrease of amplitude of contraction with 1 mg./Kg. doses of the alkaloid. In one dog a 0.25 mg./Kg. dose of pilocereine by vein slightly reduced, but did not reverse, the blood pressure response to a 15 μg. dose of epinephrine. A dose of 0.5 mg./Kg. of ergotoxine ethanesulfonate by vein did not alter the blood pressure lowering action of 1 mg./Kg. of pilocereine. Further the depressor action from 2 μg./Kg. of methacholine was not altered by 1 mg./Kg. of pilocereine. Figure 1 illustrates the action of 1 mg./Kg. of pilocereine on the dog's blood pressure and respiration.

Pilocereine acted differently upon the blood pressure and heart rate of anesthetized cats. Doses of 0.25 mg./Kg. and 0.5 mg./Kg. briefly lowered the blood pressure, but this was followed by a more prolonged rise. In nine cats the average fall of blood pressure was 20 mm. Hg for less than ten minutes, with a subsequent rise of 30 mm. Hg for less than thirty minutes. Larger doses, 1 mg./Kg., 2 mg./Kg., and 4 mg./Kg. by vein, only lowered the blood pressure for ten to thirty minutes. In all tests the heart rate increased twenty to forty-five beats per minute and the respiration rate, twelve to sixteen per minute. In one cat 10 mg./Kg. by stomach tube did not affect the blood pressure during one hour's observation. A dose of 1 mg./Kg. of atropine did not alter the blood pressure response of two cats to intravenous injection of 0.5 mg./Kg. of the alkaloid. In two other cats 0.5 to 1 mg./Kg. of pilocereine did not produce ganglionic block on the nictitating membrane preparation.

In the two rats studied 1 mg. of pilocereine raised the blood pressure 25 to 27 mm. Hg for ten minutes. Doses of 0.1 mg. and 0.2 mg. were not active. In one rat the blood pressure was raised 18 mm. Hg by 0.5 mg. of the alkaloid. Atropine in 0.1-mg. and 0.2-mg. doses by vein did not alter the pressor response of 0.5 mg. of pilocereine.

The blood pressure of roosters was consistently lowered with initial doses of pilocereine. Doses of 1 and 4 mg. lowered the blood pressure an average of 20-mm. Hg for five minutes. However, repeated

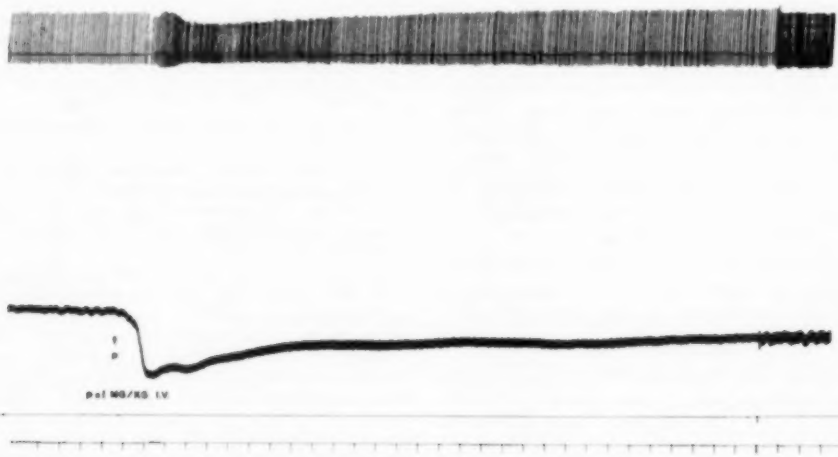

Fig. 1.—Female dog, 7.3 Kg., phenobarbital sodium anesthesia, 170 mg./Kg., i. p. Tracings from top to bottom are respiration, blood pressure, base line, and time in minutes. NOTE: Pilocereine (P) causes a fall of blood pressure. The drum was stopped at the second time mark for 30 minutes showing the hypotensive action to last more than 60 minutes. The heart rate was decreased from a normal of 120 to 92 beats per minute with a gradual recovery to 100 beats per minute at the end of one hour. The respiration rate was increased from 10 to 14 per minute, with return to normal within an hour.

and larger amounts were not active in the same animal.

Action on Isolated Smooth Muscle.—On the isolated uterus of guinea pigs and rabbits pilocereine had only a slight stimulating action with dilutions up to 1:10,000. However, the stimulating action of posterior pituitary extract on the isolated guinea pig uterus was reduced, and the epinephrine stimulation of the rabbit uterus was partially blocked by 1:10,000 dilutions of pilocereine.

Dilutions of 1:20,000 of the alkaloid induced a brief spasm of the isolated rabbit small intestine followed by a reduction of tone and amplitude. Repeated applications of the alkaloid to the same muscle were not active. The relaxing action of 1:20,000,000 dilutions of epinephrine was not altered, but spasms of the small intestine of the rabbit with 1:20,000,000 dilutions of methacholine were released by 1:10,000 dilutions of pilocereine. The muscle did not again respond to methacholine until after six or more changes of the bathing fluid. Likewise, the isolated guinea pig intestine was only slightly stimulated or relaxed with 1:10,000 and 1:20,000 dilutions of pilocereine. However, these dilutions antagonized the spasm caused by 1:10,000,000 dilutions of histamine and methacholine. Also, as observed with the isolated small intestine of rabbits, the spasm-producing agents had no effect on the isolated small intestine of guinea pigs until after as many as eight changes of Tyrode's solutions. Figure 2 shows the methacholine blocking action of pilocereine on the isolated small intestine of the guinea pig.

Fig. 2.—Action of pilocereine on a strip of a guinea pig's isolated small intestine suspended in a 100-ml. bath of Tyrode's solution at 37.5°. Note that 5 mg. of pilocereine (P) blocked the stimulating action of 10 µg. of methacholine. Also note that eight doses of methacholine five minutes apart were required before the pilocereine action was completely removed.

Action on the Perfused Frog Heart.—Dilutions of 1:7,500 and 1:10,000 of pilocereine caused a heart block, with slow recovery. Weaker dilutions, up to 1:100,000, induced a partial block of the amplitude with no change of heart rate.

Antimalarial Action in Canaries.—Eight birds, given single oral doses of 15 mg./Kg., showed a parasitic reduction equal to that produced by quinine, the "Q" value of the pilocereine being equal to 1.

Toxicity Studies in Mice.—The intravenous LD_{50} ± S.E. of pilocereine in starved albino mice was calculated to be 52 ± 4.5 mg./Kg. Immediately following the intravenous injection of toxic doses, the mice became prostrate, gasped for breath, and had kicking movements of the hind legs. The heart on exposure beat rhythmically and continued so for thirty to sixty seconds. Within ten minutes, all animals either died, or recovered completely.

DISCUSSION

Pilocereine, in the form of hydrochloride, lowers blood pressure in anesthetized dogs and roosters with acceleration of respiration when injected intravenously. There is a species difference of response. For example, the alkaloid raises blood pressure in anesthetized rats. In cats doses of 0.25 to 0.5 mg./Kg. cause a primary fall followed by a rise of arterial pressure. Repeated injections result in gradual decrease in response of blood pressure. Depression of the frog heart occurs when the organ is perfused through the inferior vena cava. Pilocereine relieves the spasm of the isolated guinea pig uterus to posterior pituitary extract or the spasm of the rabbit uterus to epinephrine. Similarly it relaxes the spasm of isolated rabbit's or guinea pig's small intestines induced by methacholine or histamine. Pilocereine seems to have a paralyzing effect on these preparations because repeated washings are required to restore their response to histamine or methacholine. If pilocereine is applied to the isolated uterus or small intestines alone, there is usually a slight stimulating action with or without a secondary depression. All in all, however, pilocereine appears to have a direct depressant action on smooth muscle organs, thus accounting for the depressor action in dogs, roosters, and cats, and antispastic action on the isolated uterus and small intestines.

One interesting feature of pilocereine is its antimalarial action in canaries infected with *P. relictum*, being almost as effective as quinine. The alkaloid is relatively toxic to mice by intravenous injection.

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The Effect of Emetine upon Rat Heart Respiration*

By RICHARD A. DEITRICH and HAROLD C. HEIM

Emetine exerted an inhibitory effect on the endogenous respiration of rat heart homogenates and on respiration of the homogenates in the presence of glucose, pyruvate, fumarate, and malate. It stimulated respiration of the homogenates in the presence of succinate and increased the activity of a succinic oxidase preparation made from rat heart. Because the oxidation of succinate is not an efficient source of energy for the heart, it seems possible that the cardiotoxic effect of emetine is in part due to the enhanced oxidation of succinate accompanied by an impaired utilization of other substrates.

THE CARDIOTOXIC effects of emetine have been studied by numerous investigators who have shown that this drug produces ventricular fibrillation (1), a diminished systolic force (2), and ventricular tachycardia (3). It is the belief of some investigators (4) that emetine disturbs, in some way, the conversion of glycogen to contractile energy in the myocardium.

Because some of the cardiotoxic effects of emetine might be due to interference with metabolic processes of the myocardium, it appeared of interest to investigate the effect of emetine upon certain respiratory processes of heart homogenates.

EXPERIMENTAL

Adult Sprague-Dawley rats of either sex were killed by decapitation and the hearts were immediately removed, opened, and washed in cold *M*/30 phosphate buffer. The buffer was *M*/30 potassium phosphate and was adjusted to *p*H 7.4. The hearts then were homogenized according to the method of Potter and Elvehjem (5) in cold buffer containing one of the following substrates, depending upon the experiment being performed: glucose, succinate, malate, fumarate, or pyruvate. The homogenates were prepared so that 1 cc. contained 100 mg. of fresh tissue. One cubic centimeter of homogenate was added to iced Warburg vessels containing 1.3 cc. of buffer in the main compartment, 0.5 cc. of emetine HCl or buffer in the sidearm, and 0.2 cc. of 10% KOH plus a pleated strip of No. 41 filter paper in the center well. The gas phase was air and the temperature of the bath was 37°. After a ten-minute equilibration period the sidearm contents were tipped into the main compartment, the manometers were closed and the oxygen consumption was read after one hour had elapsed.

Experiments were also performed in which homogenates prepared from rat spleen and rat liver replaced those prepared from heart. The liver and spleen homogenates were prepared in the same manner as those prepared from heart.

The succinoxidase preparation used in some of the experiments was made from rat heart according to the technique described by Umbreit, *et al.* (6).

The *p*H of all solutions was adjusted to 7.4 before use and the *p*H of a representative number of flasks was checked with a glass electrode instrument at the conclusion of the experiments. The final concentrations of substrates and emetine are included in Table I. For each experiment at least four animals were used and several flasks were run simultaneously with homogenate from each animal. The total number of flasks used to obtain the results is shown in Table I.

RESULTS AND DISCUSSION

The results reported in Table I indicate that emetine exerts an inhibitory effect on the respiration of rat heart homogenates in the presence of most of the substrates included in this study. When succinate was used as the substrate, however, it was found that emetine caused an increase in the oxygen uptake over that produced in the absence of emetine. Farssman and Lindsten (7) reported that succinate depresses the isolated rabbit heart. Colowick, *et al.* (8), have demonstrated that succinate oxidation is coupled with phosphorylation in heart muscle extracts and Ochoa (9) has shown that the efficiency of the coupled process is low. Furchtgott and Schorr (10) showed that succinate stimulated the oxygen uptake of heart muscle slices while depressing phosphorylation, thus producing a decrease in creatine phosphate and ATP. As a result of studies with the dog heart-lung preparation Wollenberger (11) concluded that succinate is a poor source of energy for the myocardium. In another study Wollenberger (12) noted that, in hearts poisoned with certain anesthetic agents, the oxygen uptake was depressed but, with succinate as the substrate, no depression was observed. This would appear to indicate that the anesthetic agents had impaired the ability of the myocardium to oxidize certain metabolites other than succinate and, since the oxidation of succinate does not provide as much energy as does the oxidation of other substrates, the result would be impaired efficiency of the heart.

Table I shows that the activity of succinoxidase preparations made from rat heart is increased by emetine. This would appear to correlate with the suggestion of Wollenberger (11) to the effect that the succinic oxidase system, which is apparently not inhibited by emetine, is able to compete more

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Abstracted from a thesis submitted by Richard A. Deitrich in partial fulfillment of the requirements for the degree of Master of Science in pharmacy.

TABLE I.—EFFECT OF EMETINE ON REACTIONS

Tissue	Number of Flasks	Concentration of Substrate	Concentration of Emetine	Oxygen Uptake[a]	Change, %
Heart	11	None	None	29	−36
	7		10^{-3} *M*	18	−35
	7		5×10^{-4} *M*	19	
	12	0.011 *M* Glucose	None	42	
	12		10^{-3} *M*	32	−26
	12		5×10^{-4} *M*	39	−10
	8	0.015 *M* Pyruvate	None	154	
	8		10^{-3} *M*	69	−56
	7		5×10^{-4} *M*	103	−33
	8	0.015 *M* Fumarate	None	158	
	7		10^{-3} *M*	69	−57
	9		5×10^{-4} *M*	103	−35
	8	0.015 *M* Malate	None	114	
	9		10^{-3} *M*	49	−57
	9		5×10^{-4} *M*	67	−41
	8	0.015 *M* Succinate	None	360	
	7		10^{-3} *M*	474	+24
	9		5×10^{-4} *M*	414	+13
Succinic oxidase	6	0.0085 *M* Succinate	None	130	
	6		10^{-3} *M*	161	+26
	6		5×10^{-4} *M*	144	+ 5
Liver	9	0.0085 *M* Succinate	None	345	
	9		10^{-3} *M*	334	− 3
	9		5×10^{-4} *M*	335	− 3
Spleen	8	0.0085 *M* Succinate	None	65	
	8		10^{-3} *M*	56	−29
	8		5×10^{-4} *M*	60	− 8

[a] Expressed as cu. mm. O_2/100 mg. fresh tissue.

actively with other oxidative systems for oxygen, resulting in a more intense and prolonged negative inotropic action of succinate.

The concentrations of emetine used in this study were considerably higher than the concentrations found in hearts by Gimble, Davison, and Smith (13) forty-eight hours after the intraperitoneal injection of emetine (2 mg./Kg.) into rats. They further found, however, that even after thirty-five days had elapsed, about 35% of the injected emetine was still present in the animal. It would appear, therefore, that the cumulative nature of emetine might account for cardiotoxic effects *in vivo* through prolonged effect on metabolic processes of the heart and which could conceivably involve the same mechanisms that are involved *in vitro* with higher concentrations.

Emetine has been reported to be concentrated in the liver and spleen after administration *in vivo* (13), but apparently does not damage these organs. The results shown in Table I indicate that emetine exerts an inhibitory rather than a stimulatory effect on the oxygen uptake of liver and spleen homogenates respiring in the presence of succinate.

It is not possible at this time to correlate *in vitro* observations made with heart homogenates with the *in vivo* effects of emetine. On the basis of the results of this study, however, it appears possible that emetine exerts at least a portion of its cardiotoxic effects by depressing some metabolic processes of the heart while exerting a stimulatory effect on the metabolism of succinate. Results of a study by Boyd and Scherf (14), concerning the effect of emetine on the electrocardiogram indicate that there actually is a marked disturbance in energy utilization by the heart after the *in vivo* administration of emetine.

SUMMARY

The effect of emetine on the respiration of rat heart homogenates has been investigated. Emetine was found to decrease the endogenous oxygen uptake of rat heart homogenates as well as the oxygen uptake in the presence of glucose, pyruvate, malate, and fumarate. Emetine increased the oxygen uptake of rat heart homogenates in the presence of succinate and increased the activity of a rat heart succinic oxidase preparation. It is suggested that emetine may exert at least a portion of its toxic effect upon the heart by enhancing the oxidation of succinate.

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An *in Vitro* Study of Antacids: Methods and Modifying Factors*

By J. J. HEFFERREN, G. SCHROTENBOER, and W. WOLMAN

The *in vitro* conditions reported were arbitrarily established to permit a comparison of various methods and of various media for checking antacids. The data cannot be interpreted in terms of *in vivo* conditions because of the large number of biological variables. Three widely used *in vitro* antacid evaluation methods were examined using seven representative commercial antacid preparations. Each of the three methods gave different results, although the same order of *in vitro* effectiveness is maintained regardless of the method used. A comparative study was made of various simulated gastric juices and pooled human gastric juice. The addition of mucin to U. S. P. simulated gastric juice gave a titration curve and antacid response similar to that of human gastric juice.

THE INTRODUCTION of compounds and mixtures to combat stomach acidity has been accompanied by the development of *in vitro* methods to determine their relative efficiency (1–9). The development of *in vitro* methods to evaluate the acid-neutralizing capacity of various compounds is relatively easy; however, correlation of these results with the *in vitro* results of other investigators and with *in vivo* results is very difficult.

This correlation of results is hindered in some instances by the frequently observed differences in antacid activity of the same compound between different manufacturers and between lots from the same manufacturer (1–3, 5, 6, 11, 13). The inherent differences in the methods are a result of arbitrary standardization of such factors as temperature, rate, and conditions of agitation, and composition of testing medium, all of which have been shown to modify the results (7, 10, 12). Finally, variability of *in vivo* conditions even of the same individual and the antacid needs of different individuals, all make it difficult to correlate and evaluate the results of these methods.

The purpose of this study is to compare the procedures of three *in vitro* methods widely used in determining the antacid activity, and to study the effect of several testing media and certain of their components. The tests were carried out on seven representative commercial antacid tablet preparations: dried aluminum hydroxide gel, 1 part aluminum hydroxide and 2 parts magnesium trisilicate, 2 parts aluminum hydroxide and 1 part magnesium trisilicate, aminoacetic acid and calcium carbonate, dihydroxy aluminum aminoacetate, dihydroxy aluminum sodium carbonate, and hydroxy aluminum magnesium aminoacetate.

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Recently Booth and Dale (12) reported data of a somewhat similar study in which they compared the results of nine *in vitro* methods using antacid mixtures representing fast, slow, and combination of fast and slow acting antacids.

PROCEDURES

General.—Accurately weigh at least twenty-five antacid tablets and finely grind them with a mortar and pestle. An accurately weighed amount of this powdered material equivalent to the weight of the maximum dose recommended on the label was used for testing. This dose was used in all cases except two where an intermediate dose was used for comparative reasons. Automatic pipets with a fairly large tip bore and calibrated for 5 ml. were used for transferring the testing medium.

Dale and Booth (2).—Using the same general procedure, the maximum dose recommended on the label was added to 50 ml. of 0.1 *N* HCl. The *p*H was taken at the end of one, three, five, and ten minutes and then every ten minutes. After each ten-minute *p*H reading, 2 ml. of 1 *N* HCl was added. This step was repeated until a *p*H of 1.8 was reached.

Hammarlund and Rising (3).—Using the same general procedure, the maximum dose recommended on the label was added to 25 ml. of 0.1 *N* HCl. The *p*H was read every five minutes for the first thirty minutes and then every thirty minutes. After each thirty-minute *p*H reading, 25 ml. of 0.1 *N* HCl at 37° was added. The test was run for three hours.

Johnson and Duncan (1).—Using the same general procedure twice the maximum dose recommended on the label was added to 100 ml. of 0.1 *N* HCl at 37°. The *p*H was taken at one, two, three, five, and ten minutes and then every ten minutes. After each ten-minute *p*H reading, 20 ml. of the reaction mixture was removed and 20 ml. of 0.1 *N* HCl at 37° added. This procedure was repeated until a *p*H of 1.8 was reached.

Modified Johnson and Duncan.—To 50 ml. of testing medium at 37° the maximum dose recommended on the label of antacid was added. The *p*H of the mechanically stirred mixture was determined at one, two, three, and five minutes and at five-minute intervals thereafter. Immediately after

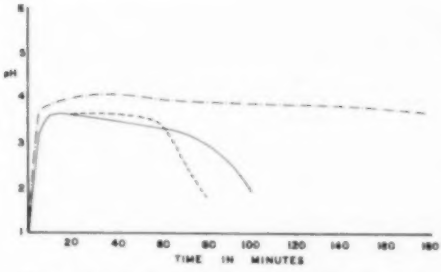

Fig. 1.—Activity of antacids by three commonly used *in vitro* methods. Antacid D. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and — — Dale & Booth.

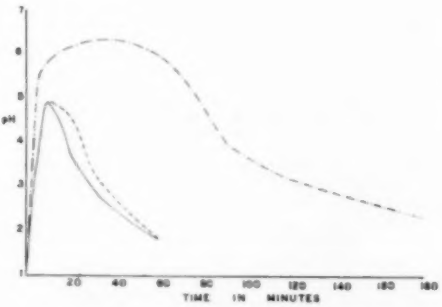

Fig. 2.—Activity of antacids by three commonly used *in vitro* methods. Antacid A. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and —— Dale & Booth.

each five-minute *p*H reading, 5 ml. of the reaction mixture was removed and 5 ml. of fresh medium at 37° quickly added with automatic pipets. This procedure was repeated until a *p*H of 1.8 was reached.

DISCUSSION

The available *in vitro* methods may be divided into two general classes: those involving periodic partial removal of the reaction mixture and an equivalent addition of fresh medium and those involving only periodic addition of fresh medium. Two of the methods chosen for comparison, those of Hammarlund and Rising (3) and Dale and Booth (2) are of the latter classification; however, they differ significantly in the rate and the number of equivalents of acid added to the reaction mixture. At the end of sixty minutes, the method of Hammarlund and Rising has had a total of 5 mEq. of acid added, whereas the method of Dale and Booth has had a total of 17 mEq. of acid added. The third method, that of Johnson and Duncan (1) involves periodic partial removal of the reaction mixture and addition of fresh medium. The procedure of Johnson and Duncan involves the addition of 22 mEq. of acid in sixty minutes to twice the dose used in the other two methods.

Figures 1 to 7 demonstrate the variable results obtainable even when a number of variables such as lot-to-lot differences, temperature, rate of stirring, particle size, and other physical aspects are constant. When the method of Hammarlund and Rising was used, six out of the seven antacid preparations had a *p*H of 3–4 at the end of the test period of three hours, indicating little differentiation of the antacids at this time. The method of Dale and Booth gave results somewhat similar to the method of Johnson and Duncan.

The results show differences not only characteristic of the antacid under test but also of the method used; however, the same order of effectiveness shown by the three methods for a given antacid was maintained for all antacids.

The three methods chosen utilize 0.1 *N* HCl exclusively as a testing medium with the partial exception of the method of Dale and Booth where 1 *N* HCl was used for subsequent renewal of the acid in the reaction mixture. Ingredients such as pepsin, peptone, and sodium chloride have been included as constituents of simulated gastric media to relate it more closely to human gastric juice (6, 8, 9–11); however, others have stated that such an addition has little effect on the end result of the testing program (3, 7).

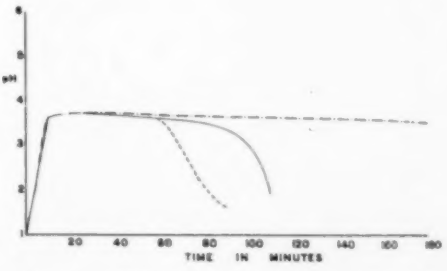

Fig. 3.—Activity of antacids by three commonly used *in vitro* methods. Antacid G. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and —— Dale & Booth.

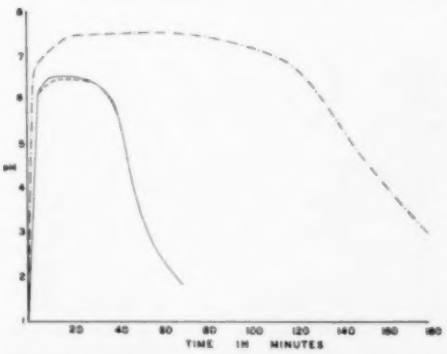

Fig. 4.—Activity of antacids by three commonly used *in vitro* methods. Antacid B. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and —— Dale & Booth.

Table I shows the compositions of simulated gastric juice, U. S. P. XV and the simulated gastric juice used for several years in the Chemical Laboratory of the A. M. A. for tests and standards (T. & S. juice). This T. & S. juice has been abandoned because of the inclusion in the U. S. P. of an official simulated gastric juice.

TABLE I.—SIMULATED GASTRIC JUICE

	U. S. P.	T. & S.[a]
Pepsin	3.2 Gm.	3.0 Gm.
Sodium chloride	2.0 Gm.	2.0 Gm.
HCl, conc.	7.0 ml.	5.3 ml.
Mucin	.....	5.0 Gm.
KH_2PO_4, 0.2 *M*	.....	37.0 ml.
Water, *q. s.*	1,000 ml.	1,000 ml.
	*p*H 1.2	*p*H 1.4

[a] This formulation developed primarily by Mr. H. J. Rynkiewicz for our laboratory in 1949 and used since that time for tests and standards work.

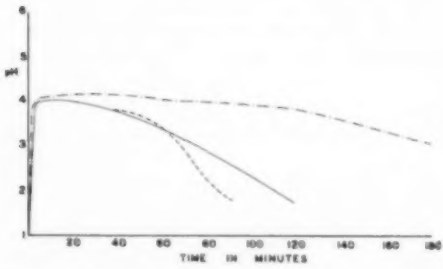

Fig. 5.—Activity of antacids by three commonly used *in vitro* methods. Antacid C. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and —— Dale & Booth.

Recently a study (not reported) was made in our laboratory on the effects of these two media on various antacids using our modified procedure of Johnson and Duncan. It was observed that usually the antacid in use brought the U. S. P. simulated gastric juice to a higher *p*H than it did the T. & S. simulated gastric juice despite the fact that the U. S. P. juice was originally lower in *p*H. The primary difference between the two juices other than the *p*H is the presence of mucin and KH_2PO_4 in T. & S. juice. In order to determine the effects on U. S. P. juice of mucin and potassium dihydrogen phosphate, they were individually added to the U. S. P. juice and the resulting mixture adjusted to *p*H 1.2. All of these media were then compared with samples from 6 L. of pooled human gastric juice having a *p*H of 1.4.[1]

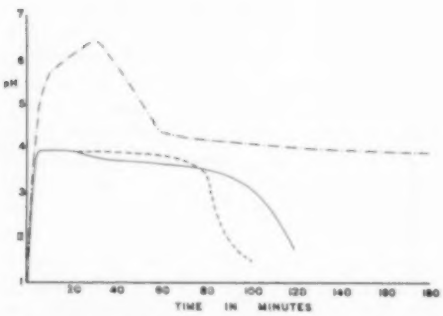

Fig. 6.—Activity of antacids by three commonly used *in vitro* methods. Antacid F. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and —— Dale & Booth.

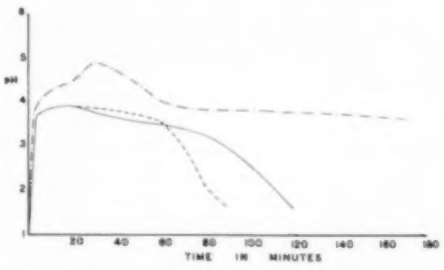

Fig. 7.—Activity of antacids by three commonly used *in vitro* methods. Antacid E. —·— Hammarlund & Rising, – – – – Johnson & Duncan, and —— Dale & Booth.

In Fig. 8 the potentiometric titrations of the pooled human gastric juice may be compared with T. & S. juice, U. S. P. juice, and the modified U. S. P. juices. It means little to compare these titration curves at their equivalence points since this will largely reflect the difference in the total number of

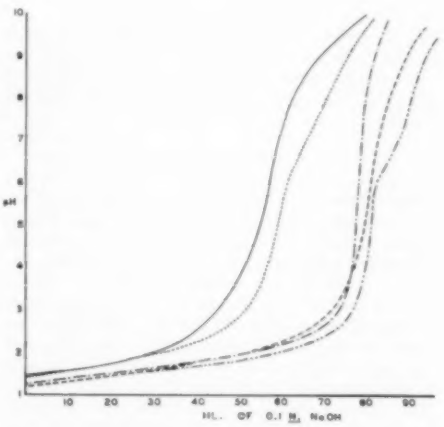

Fig. 8.—Potentiometric titration of 100 ml. of various gastric media. —— human, pooled, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —·—·— U. S. P. + KH_2PO_4, *p*H 1.2; – – – U. S. P. + mucin, *p*H 1.2; and . . . . . T & S, simulated, *p*H 1.4.

[1] The authors are indebted to the Surgical Clinical Laboratory, Billings Hospital, University of Chicago, and in particular to Dr. Lester Dragstedt and Mrs. Elizabeth Pravatiner for collecting and supplying the human gastric juice.

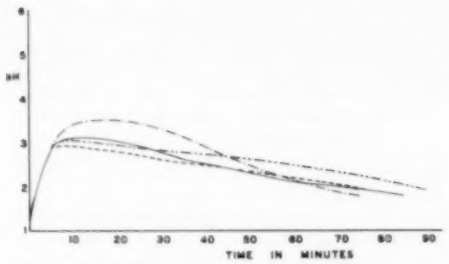

Fig. 9.—Activity of Antacid D using various gastric media with the modified method of Johnson and Duncan. —— human, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —··— U. S. P. + KH_2PO_4, *p*H 1.2; and – – – U. S. P. + mucin, *p*H 1.2.

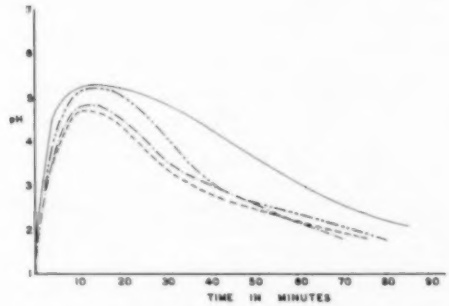

Fig. 10.—Activity of Antacid A using various gastric media with the modified method of Johnson and Duncan. —— human, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —··— U. S. P. + KH_2PO_4, *p*H 1.2; and – – – U. S. P. + mucin, *p*H 1.2.

equivalents of acid present in the juice; rather, the total shape of the curves is an indication of the nature of the constituents and their buffering effects. Thus from Fig. 8 it can be seen that the addition of mucin to the U. S. P. juice alters the shape of the U. S. P. curve so that it is more closely related to that of the human gastric juice.

In addition to the potentiometric titration, comparison of the action of the different juices was made with regard to antacid preparations (Figs. 9–14) using the modified procedure of Johnson and Duncan. First of all it is necessary to remember that the pooled human juice used had a *p*H of about 1.4 whereas the U. S. P. and modified U. S. P. juices

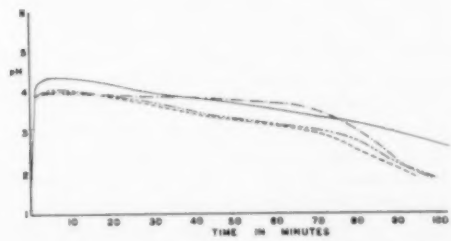

Fig. 11.—Activity of Antacid F using various gastric media with the modified method of Johnson and Duncan. —— human, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —··— U. S. P. + KH_2PO_4, *p*H 1.2; and – – – U. S. P. + mucin, *p*H 1.2.

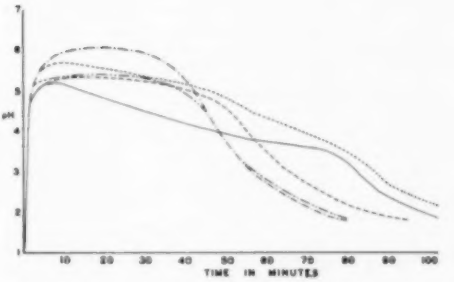

Fig. 12.—Activity of Antacid B using various gastric media with the modified method of Johnson and Duncan. —— human, *p*H 1.4; . . . . . human, filtered, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —··— U. S. P. + KH_2PO_4, *p*H 1.2; and – – – U. S. P. + mucin, *p*H 1.2.

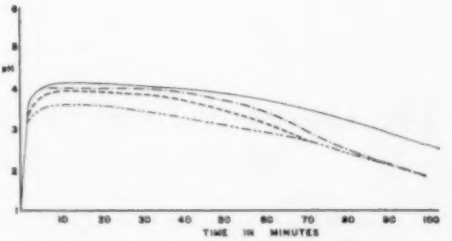

Fig. 13.—Activity of Antacid C using various gastric media with the modified method of Johnson and Duncan. —— human, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —··— U. S. P. + KH_2PO_4, *p*H 1.2; and – – – U. S. P. + mucin, *p*H 1.2.

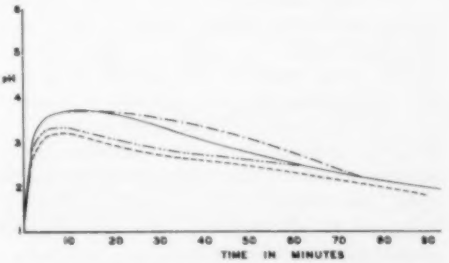

Fig. 14.—Activity of Antacid E using various gastric media with the modified method of Johnson and Duncan. —— human, *p*H 1.4; —·— U. S. P., simulated, *p*H 1.2; —··— U. S. P. + KH_2PO_4, *p*H 1.2; and – – – U. S. P. + mucin, *p*H 1.2.

had a *p*H of 1.2. In all cases except that of the antacid B (Fig. 13), filtration of the human juice had little or no effect. There was also little difference between the titration curves of the filtered and unfiltered human gastric juice; however, it was much

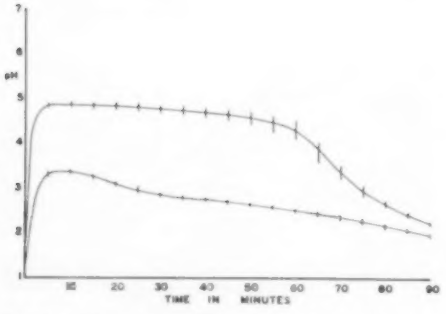

Fig. 15.—Reproducibility of modified method of Johnson and Duncan.

easier to work with the filtered human juice. The addition of mucin or potassium dihydrogen phosphate to the U. S. P. juice in general had about the same effect, that is, it lowered the maximum *p*H value and somewhat prolonged the antacid effectiveness. This is what might be expected with the addition of a buffer.

The reproducibility of the modified method of Johnson and Duncan using various synthetic gastric testing media is dependent upon several factors including the individual chemical nature and particle size of the antacid as well as the mechanics of the method and accuracy in preparing the testing media. Figure 15 represents the variation in the results obtained with the modified method of Johnson and Duncan using three different batches of the same testing media. The curves in this figure represent two antacid preparations distinctly different in composition and *in vitro* activity.

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The Use of Sodium Tetraphenylboron as a Means of Identifying and Isolating Alkaloids*

By WILLIAM E. SCOTT, HARRY M. DOUKAS, and PAUL S. SCHAFFER

The use of a new reagent, sodium tetraphenylboron, as a means of identifying alkaloids is described. Methods are given for isolating alkaloid complexes and for determining their physical constants as an aid in the identification of the alkaloids. A simple method of recovery of the alkaloids is also given.

THIS LABORATORY has been conducting a search for new alkaloids from plants for use as medicinals, antifungal agents, or insecticides, with a view to the possible development of new cultivated crops as a source of income for the farmer. The screening followed the usual pattern of extracting dry plant material with a suitable solvent, such as 85 per cent methanol or ethanol, concentrating the solution under vacuum, dissolving the residue in slightly acidic water, and testing for the presence of alkaloids. Solutions of silicotungstic acid and Mayer's reagent were used for the test.

* Received April 3, 1956, from the Eastern Regional Research Laboratory, Philadelphia 18, Pa., a laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.
The authors wish to acknowledge the assistance of Mr. J. S. Ard in making the nitrogen determinations.

Schultz and Mayer (1) have described the use of a new reagent for the qualitative and quantitative determination of nitrogen-containing medicinals, which included alkaloids. The reagent was known as "Kalignost," sodium tetraphenylboron. They used an aqueous solution of this reagent to precipitate alkaloids from a slightly acidic solution which had been heated to 70°. Fischer and Karawia (2) listed the melting points of 17 alkaloid complexes of sodium tetraphenylboron which they had prepared. More recently, Barnard (3) published a comprehensive bibliography on the uses of sodium tetraphenylboron as a complexing agent.

We prepared solutions of thirty-four known alkaloids of various structures, in order to make a comparison of the sensitivity of Mayer's

reagent and sodium tetraphenylboron toward alkaloids, by observing the amount of precipitate obtained with these reagents. We also investigated the possibility of using the precipitate obtained with sodium tetraphenylboron as a means of isolating the free alkaloid by gently heating the complex with dilute alkali. It was possible, in most cases, for us to regenerate the alkaloid by this method.

We prepared sodium tetraphenylboron derivatives of twenty-one known alkaloids to aid us in the identification of alkaloids from the new plant sources. The derivatives were purified by crystallization from suitable solvents, melting points were recorded, and Kjeldahl nitrogen analyses were made to check on the purity of the compounds. The free alkaloids were recovered by heating the complexes in the presence of alkali, except in a few cases where it was impossible to recover the alkaloid due to unfavorable conditions.

EXPERIMENTAL

Materials.—The reagents used were sodium tetraphenylboron (purchased from J. T. Baker Chemical Co.[1]) and Mayer's reagent. The alkaloids were of commercial grade, with the exception of the tomatine derivatives, nicotine, solanthrene, solanine, and solanidine, which were prepared in this laboratory.

Comparison of Sodium Tetraphenylboron with Mayer's Reagent in the Detection of Alkaloids.—Duplicate samples of approximately 20 mg. of alkaloid were dissolved in 15 cc. of water. To one set of samples, 6 drops of glacial acetic acid was added and the solutions were heated to 70° on the steam bath. A 0.1 *M* solution of sodium tetraphenylboron in water was added to the heated samples until no further precipitation occurred. To the other set of samples, 5 drops of a 15% hydrochloric acid solution was added. Then a slight excess of Mayer's reagent was added. Visual comparisons of the amount of precipitate or turbidity were made for each set of samples. A designation of 3 was given for a heavy precipitate, 2 for a medium precipitate, 1 for a turbid solution and 0 for no precipitate. The results are listed in Table I.

Preparation of Complex for Analysis.—Approximately 0.5-Gm. samples of alkaloid were dissolved in a small quantity of water which had been made slightly acid with acetic acid. The solutions were heated to 70° on the steam bath. An equimolar solution of sodium tetraphenylboron plus a 0.1-Gm. excess in water was added to the warm alkaloid solution and the precipitate was allowed to settle. The precipitate was recovered by filtration and dried in a vacuum oven. The precipitate was weighed, then recrystallized from either acetone or ethanol. Melting points were determined on a Kofler Micro Hot Stage with a calibrated thermometer. Kjeldahl nitrogen determinations were made on all compounds.

[1] Mention of firms or trade names does not imply endorsement of the U. S. Department of Agriculture over similar firms or products not mentioned.

The alkaloid was regenerated by the addition of a 5% solution of sodium hydroxide to the solution of the complex dissolved in either acetone or ethanol. The solutions were heated gently on the steam bath for 15 minutes. The solvent was removed by means of an air stream and the residue was extracted with chloroform. The chloroform after being washed with water to remove alkali, was dried and then concentrated. The recovered alkaloids were weighed and identified by means of melting points. The results are shown in Table II.

TABLE I.—A COMPARISON OF THE SENSITIVITY OF SODIUM TETRAPHENYLBORON AND MAYER'S REAGENT TOWARD ALKALOIDS

Alkaloid	Sodium Tetraphenylboron[a]	Mayer's Reagent[a]
Aconitine	2	2
Agaricin	1	1
Aspidospermine	2	2
Atropine	3	3
Berberine HCl	3	3
Betaine	2	0
Brucine	3	1
Cinchonidine sulfate	2	2
Cinchonine sulfate	2	3
Cocaine	3	3
Codeine	3	3
Colchicine	1	2
Dihydrotomatidine	1	1
Emetine HCl	3	2
Ephedrine sulfate	1	0
Homatropine HCl	3	1
Hydrastine HCl	3	3
Hyoscyamine	1	1
Jervine	3	3
Lobeline sulfate	3	3
N-Acetyltomatidine	1	0
Physostigmine	2	2
Pilocarpine HCl	3	2
Quinidine sulfate	3	3
Quinine sulfate	3	3
Sanguinarine nitrate	2	1
Scopolamine HBr	2	1
Solanidine	2	2
Solanine	1	0
Solanthrene	1	1
Sparteine sulfate	2	3
Tomatidine	2	0
Tomatine	1	0
Yohimbine HCl	3	3

[a] 3 = heavy precipitate; 2 = medium precipitate; 1 = turbid solution; 0 = no precipitate.

DISCUSSION

In comparing the sensitivity of Mayer's reagent and sodium tetraphenylboron toward the alkaloids listed in Table I, it will be noted that at the concentrations which we were using, Mayer's reagent failed to show any turbidity with ephedrine, solanine, tomatidine, tomatine, N-acetyltomatidine, or betaine. In the other solutions tested, the use of sodium tetraphenylboron was as good as or superior to Mayer's reagent. Since sodium tetraphenylboron will also precipitate potassium and ammonium ions out of solution, it is necessary that the use of re-

TABLE II.—CHARACTERISTICS OF ALKALOID-SODIUM TETRAPHENYLBORON COMPLEXES

Alkaloid	Yield of Complex, %	M. p. of Complex, °C.	Nitrogen, % Actual	Nitrogen, % Theoretical	Regenerated Alkaloid Recovery, %
Aspidospermine	84.5	126–8	4.32	4.15	72.6
Atropine	97.4	141–4	2.36	2.30	60.8
Berberine HCl	68.4	204–7	2.12	2.13	....
Brucine	94.4	156–8	3.82	3.91	90.8
Cinchonidine sulfate	60.0	133–5	4.40	6.37	97.5
Cinchonine sulfate	55.6	211–4	5.82	6.17	100
Colchicine	49.2	100–5	2.30	1.78	8.3
Emetine HCl	80.6	111–4	2.73	3.50	85.6
Ephedrine sulfate	97.0	135–8	2.83	2.89	100
Homatropine HCl	97.4	151–4	2.34	2.36	3.8
Hydrastine HCl	92.6	109–11	2.00	1.99	76.2
Hyoscyamine	98.2	142–5	2.33	2.30	15.0
Jervine	78.4	125–6	1.88	1.91	100
Nicotine	100	131–3	6.04	5.82	98.4
Protopine	100	130–5	1.95	2.08	100
Quinine sulfate	87.8	163–5	4.23	4.34	100
Sanguinarine nitrate	73.8	140–3	2.19	2.09	78.5
Scopolamine HBr	95.0	145–8	2.26	2.24	15.2
Sparteine sulfate	100	205–8	4.90	5.06	100
Tomatidine	84.8	129–31	1.88	1.90	99.1
Yohimbine HCl	91.2	159–61	3.98	4.17	2.4

agents or solutions containing these ions be avoided.

The yields of the alkaloid complexes were fairly high, as shown in Table II. The melting points in some cases overlap, precluding their use in the identification of the alkaloid without additional information. The amount of alkaloid which we recovered was, in some cases, very low. This may be due to overheating or to the use of too strong an alkali solution.

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Preparation of Bis-(2-Nitro-2-Methylpropyl)-Sulfite*

By WAYNE KESSLER, M. I. BLAKE, and C. E. MILLER

Bis-(2-nitro-2-methylpropyl)-sulfite has been prepared by the controlled reaction between 2-nitro-2-methylpropanol-1 and thionyl chloride. This new nitro sulfite ester has been characterized by oxidation to the corresponding sulfate and its ultraviolet absorption spectrum has been determined. The results of screening of this compound on Sarcoma 180 *in vivo* inhibition are reported.

PREVIOUS PUBLICATIONS (1, 2) from this laboratory have reported the preparation of aliphatic nitro compounds for screening in connection with our cancer research program. The need for aliphatic compounds containing sulfur in an intermediate valence as well as a nitro group for a problem involving cellular oxidation-reduction studies prompted the work to be reported in this paper. A survey of the literature failed to disclose this type of compound. Lingo (3) has reported the preparation of certain cyclic nitro sulfite esters. We wish to report our method of preparation of the hitherto unknown bis-(2-nitro-2-methylpropyl)-sulfite. A typical run is described herewith.

EXPERIMENTAL

Bis - (2 - nitro - 2 - methylpropyl) - sulfite.—The reactor used in this work was a 500-cc. four-necked flask fitted with mechanical stirrer, thermometer, dropping funnel, and reflux condenser. The flask was charged with 60 Gm. (0.503 mole) of recrystallized 2-nitro-2-methylpropanol-1[1] dissolved

* Received March 9, 1956, from the School of Pharmacy, North Dakota Agricultural College, Fargo.
Presented to the Scientific Section, A.PH.A., Detroit meeting, April, 1956.
Acknowledgment is given to the North Dakota Cancer Society for financial assistance which made this work possible.
The authors wish to express their appreciation to Dr. C. Chester Stock of the Sloan-Kettering Institute for the screening of this compound.

[1] Generously supplied by Commercial Solvents Corporation.

in 200 cc. of chloroform. To this stirred solution of the nitroalcohol was added dropwise with stirring over a period of three hours 30 Gm. (0.252 mole) of purified thionyl chloride diluted with 100 cc. of chloroform. The temperature during the addition of the thionyl chloride was maintained at about 25°. After the addition of the thionyl chloride the temperature was slowly raised to reflux and maintained at slow reflux for about eight hours. The reaction mixture was cooled to room temperature, the chloroform removed by evaporation, and the resulting solid repeatedly recrystallized from acetone-water to yield 54 Gm. (75.4%) of colorless crystals.

A sample recrystallized from acetone-water for analytical use melted at 83–84°.

Anal.—Calcd. for $C_8H_{16}N_2O_7S$: C, 33.80; H, 5.67; N, 9.86; S, 11.28. Found: C, 33.89; H, 5.61; N, 9.66; S, 11.24.

This new nitrosulfite was insoluble in water, and soluble in acetone, ether, chloroform, and 1,4-dioxane. It was found to be slightly soluble in propylene glycol and propylene carbonate. The nitro sulfite ester has an ultraviolet absorption maximum at 278 mμ (Fig. 1). The molar extinction

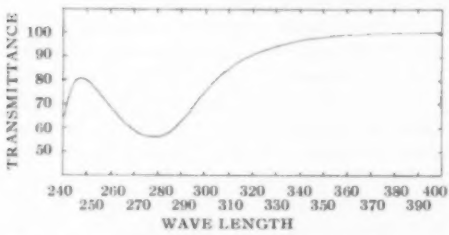

Fig. 1.—Ultraviolet spectrum of bis-(2-nitro-2-methylpropyl)-sulfite.

coefficient was found to be 46.125. Further characterization involved the formation of the corresponding sulfate following a modification of the method described by Adams (4) for preparing *p*-chlorobenzoic acid.

Bis-(2-nitro-2-methylpropyl)-sulfate.—A solution of 2 Gm. of bis-(2-nitro-2-methylpropyl)-sulfite in 100 cc. of acetone was treated with an excess of solid potassium permanganate. The mixture was refluxed on a steam bath for thirty minutes, diluted with 300 cc. of water, and sufficient sodium bisulfite added to decompose the excess potassium permanganate. The mixture was filtered and the filtrate worked up in the usual manner. Repeated recrystallization from acetone-water yielded platelets melting at 146.5–147.5°.

Anal.—Calcd. for $C_8H_{16}N_2O_8S$: C, 32.00; H, 5.38; N, 9.33; S, 10.67. Found: C, 31.73; H, 5.21; N, 9.09; S, 10.43.

LD_{50} Determination.—LD_{50} values for bis-(2-nitro-2-methylpropyl)-sulfite were determined on week-old chicks. By intramuscular administration in 1,4-dioxane the LD_{50} was found to be 630 mg./Kg. with limits of error of 97–103% (5). Toxicity studies on 1,4-dioxane administered intramuscularly showed that up to 5 cc./Kg. of body weight could be injected without untoward effect.

Sarcoma 180 in Vivo Inhibition.—The results of screening tests for bis-(2-nitro-2-methylpropyl)-sulfite as reported by the Sloan Kettering Institute are given in Table I.

Table I.—SARCOMA 180 *in Vivo* INHIBITION

Mg./Kg. Injected/ Mouse/Day	Wt. Change, Gm.[a]	Deaths
125	+1.5 / +5.0	0

[a] The numerator indicates the average weight changes in the five test animals and the denominator indicates the average weight change in the control group.

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Increased Charge for Stain Certification

The Board of Trustees of the Biological Stain Commission has found it necessary to increase the price of Certification Labels by 5¢ effective September 1, 1956. This will result in a change from the current charge of 15¢ to a new charge of 20¢ per bottle to the purchasers of Certified Stains. This price increase has been necessitated by increasing costs in the operation of the Commission, and is the first such increase in almost 20 years.

The Synthesis of Some Cyclic Acetal and Ketal Derivatives of Some Hydroxyl-Containing Compounds*

By ROBERT V. PETERSEN† and OLE GISVOLD

The following compounds, i. e., dihydropyran (I), anhydro-δ-aceto-*n*-butyl alcohol (III), anhydro-γ-aceto-*n*-propyl alcohol (VI), and 3-methyl-2,3-dihydrofuran (VIII) formed cyclic acetals (or ketals) with a secondary alcohol (cholesterol and other compounds). (I), and δ-hydroxy-valeraldehyde (II) reacted with an aromatic thiol compound (2-naphthalenethiol) to give the same mixed acetal. (I), (II), δ-aceto-*n*-butyl alcohol (IV), 3-hydroxy-δ-aceto-*n*-butyl alcohol (V), and γ-aceto-*n*-propyl alcohol (VII) all reacted with a primary alcohol (Prenderol) to produce cyclic acetals and ketals. (I), (II), and (III) also reacted with phenols (diethylstilbestrol and hexestrol) to form the corresponding cyclic acetals and ketals. The stability of the products varied widely. In general, the derivatives of phenols were less stable than the corresponding derivatives of primary and secondary alcohols, and the derivatives containing a furanose ring system were less stable than those containing a pyranose ring system.

THE PROCEDURES USED to synthesize some cyclic acetals and ketals from the reactions of certain γ- and δ-hydroxy aldehydes and ketones, and with stilbestrol, hexestrol, Prenderol®, 2-naphthalenethiol, and some steroids are described. γ- and δ-Hydroxy aldehydes and ketones exist in equilibrium with their cyclic hemiacetal and hemiketal forms (1, 2, 3). In the presence of a trace of acid as a catalyst, and under anhydrous conditions, these cyclic hemiacetals and hemiketals were allowed to react with primary and secondary alcohols, thioalcohols, and phenols to form cyclic acetals and ketals.

The usefulness of many pharmaceutical products is enhanced by making derivatives which have improved stability and solubility characteristics, both from the standpoint of dosage forms and of absorption and distribution in the body. In the case of the cardiac glycosides, such as digitoxin and digoxin, the sugar moities seem to have a pronounced influence on distribution within the body. Chen states that "it has been shown on several occasions that the aglucones of the cardiac glycosides possess a lower potency than the parent glycoside," and that "the sugar molecule, or molecules, are not absolutely necessary for the building of the most potent products" (4). This statement can be substantiated by a careful study of the intravenous potencies of a large number of naturally occurring cardiac glycosides, their degradation products, and some synthetic cardiac glycosides and a series of aliphatic acid esters of the cardiac glycosides.

It was considered worth while to synthesize other derivatives of the aglycones of the cardiac glycosides, using substituents in which the solubility of the molecule could be varied. With a variance in the solubility of the molecule, absorption, distribution, and duration of action also may be varied to an advantage.

With this purpose in mind, some derivatives of digitoxigenin and digoxigenin and of several other hydroxyl containing compounds were prepared. The cyclic acetals and ketals which were prepared are comparable to the glycosides themselves, as both are acetals and ketals formed from dihydropyran and its derivatives are analogous to aldo- and ketopyranosides; while those from derivatives of dihydrofuran are analogous to aldo- and ketofuranosides. Several of the compounds were synthesized solely to determine the reactivity of the various reagents toward "model" hydroxy compounds, without intention of producing useful pharmaceutical agents. In some cases inert solvents were used as reaction media, whereas in other cases, one of the reactants also served the purpose of a solvent.

When 2-dihydropyran (I) is allowed to react with a hydroxy-compound in the presence of an acid catalyst, and under anhydrous conditions, the resultant product (Ia) is a cyclic acetal derivative of the hydroxy-compound. This is comparable to the reaction of glycals with methanol (5, 6). When (I) is allowed to react with water, with acid as a catalyst, the product is

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δ-hydroxyvaleraldehyde (II) (1, 7, 8). Under similar conditions used to react (I) with hydroxy-compounds, (II) can be condensed with some hydroxy-compounds to give the same type product. Other derivatives of dihydropyran and δ-hydroxyvaleraldehyde react similarly with hydroxy-compounds to form analogous cyclic acetals and ketals. Anhydro δ-aceto-*n*-butyl alcohol (III), δ-aceto-*n*-butyl alcohol (IV), and 3-hydroxy-δ-aceto-*n*-butyl alcohol (V) were synthesized and used to prepare other derivatives.

The derivatives of dihydrofuran which were employed in synthesizing derivatives of hydroxy-compounds were anhydro-γ-aceto-*n*-propyl alcohol (VI), γ-aceto-*n*-propyl alcohol (VII), and 3-methyl-2,3-dihydrofuran (VIII).

EXPERIMENTAL

Synthesis of δ-Hydroxyvaleraldehyde (II).—A practical grade of dihydropyran (Eastman Kodak Co.) was purified by the method outlined in "Organic Syntheses" (9), and (II) was prepared from it by the method of Schniepp and Geller (1).

Synthesis of δ-Aceto-*n*-butyl Alcohol (IV) and Anhydro-δ-aceto-*n*-butyl Alcohol (III).—Four (IV) was synthesized from the sodium salt of ethyl acetoacetate and 1,3-dibromo propane in the same manner as reported by Lipp (10, 11) as modified by Bergmann and Miekeley (12). By the controlled heating of (IV) to 155–160°, it dehydrates to (III). This was carried out by heating (IV) and passing the vapors through a helices-packed column into a condensor, where a condensate of a mixture of (III) and water was collected into a separatory funnel. Three (III) was redistilled. By allowing (III) to stand for several days in the presence of acidified water, a pure grade of (IV) was formed, which was then dried in a desiccator over sulfuric acid.

Synthesis of 3-Hydroxy-δ-aceto-*n*-butyl Alcohol (V).—The oxidation of anhydro-δ-aceto-*n*-butyl alcohol with a moist ethereal solution of perbenzoic acid was carried out in a manner similar to that used by Bergmann and Miekeley (12). Perbenzoic acid was prepared from benzoyl peroxide (Lucidol Co.) and sodium methoxide according to the method described by Tiffeneau (13). A mixture of dry ice and acetone was used to control the temperature of the reaction mixture.

Synthesis of Anhydro-γ-aceto-*n*-propyl Alcohol (VI) and γ-Aceto-*n*-propyl Alcohol (VII).—(VII) was synthesized from the sodium salt of ethyl acetoacetate and 1,2-dibromoethane (Eastman Kodak Co.) by the method of Lipp (14). The use of two molecular quantities of ethylacetoacetate was used as in the synthesis of (IV) as suggested by Bergmann and Miekeley (12). Heating time of the alkylated ethyl acetoacetate with aqueous HCl was increased to twelve hours as suggested by Linnell and Melhuish (3).

Careful control of temperature was necessary to dehydrate (VII) with the formation of (VI). At a temperature of 207–208° the dehydration occurred and the products were first passed through a condensor into a separatory funnel which was immersed in ice water to retard the re-formation of (VII). After separation of (VI) from the water, formed by dehydration of (VII), (VI) was redistilled and collected for use. Pure (VII) was obtained by allowing (VI) to stand in the presence of very slightly acidified water for one day followed by drying the product over sulfuric acid.

Purification of 3-Methyl-2,3-dihydrofuran (VIII).—(VIII) was synthesized by Parham (15) and was donated for use in this work. Three cubic centimeters of (VIII) was redistilled and collected for use.

Synthesis of 2,2-Diethyl-1,3-bis-(2′-tetrahydropyranyloxy)-propane (IX).—Crystalline 2,2-diethyl-1,3-propanediol[1] 1.32 Gm. (0.1 mole) was dissolved in 6 cc. of chloroform. To this solution was added 2.10 Gm. (0.025 mole) of dihydropyran (I) followed by one drop of a 5% solution of hydrogen chloride in dioxane purified by the method of Fieser (16). The temperature of the reaction mixture raised spontaneously from 24° to 36°, and external heat was applied to increase the temperature to 50° for five minutes. The mixture was allowed to cool slowly to room temperature, and was then made slightly alkaline with 0.25 cc. of ethanolic potassium hydroxide and the solvent was removed by evaporation. The residue was distilled at 5 mm. pressure, and the fraction between 150° and 165° was collected. Four subsequent redistillations yielded a product boiling at 159–164° at 5 mm. pressure, which was collected and dried over P_2O_5 at 10 mm. pressure and at room temperature for two days. Yield, 88%.

Anal.—Calcd. for $C_{17}H_{32}O_4$: C, 68; H, 10.7. Found: C, 67.7; H, 10.5.

An identical product was obtained from 1.32 Gm. (0.01 mole) of Prenderol® and 2.55 Gm. (0.025 mole) of δ-hydroxyvaleraldehyde (II) under similar conditions. The excess δ-hydroxyvaleraldehyde was used as the solvent and the mixture was heated to 100° for two minutes.

Synthesis of 3β-2′-Tetrahydropyranyloxy-Δ⁵-cholestene (X).—When this compound was synthesized as described by Greenhalgh, *et al.* (17) from dihydropyran (I) and cholesterol, using chloroform as the solvent a 94.8% yield was obtained. Substitution of other solvents for chloroform gave the following yields, i. e., from dioxane 92.6%, from dichloromethane 89.8%, and from ethyl acetate 96.4%.

An identical product was obtained when 1.933 Gm. (0.005 mole) of cholesterol and 1.02 Gm. (0.01 mole) of δ-hydroxyvaleraldehyde (II) in 7 cc. of dioxane plus one drop of a 5% solution of hydrogen chloride in dioxane was heated to 50° for one hour. The reaction mixture, when allowed to cool to 20° and then cooled to 15°, gave crystalline material which after two recrystallizations from ethyl acetate melted at 157–158.5° and gave no depression in melting point when mixed with the product obtained from dihydropyran and cholesterol.

Synthesis of 3β-2′-Tetrahydropyranyloxy-14β-hydroxy-20-(22)-cardenolide (XI).—Two grams of digitoxin[2] was hydrolyzed by the method of Elderfield (18). The digitoxigenin thus obtained was recrystallized once from 40% aqueous ethanol, and twice from ethyl acetate yielding 510 mg. (52.2%) of digitoxigenin, m. p., 253–255°.

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[1] Prenderol-Squibb.
[2] Hosco Chemical Co.

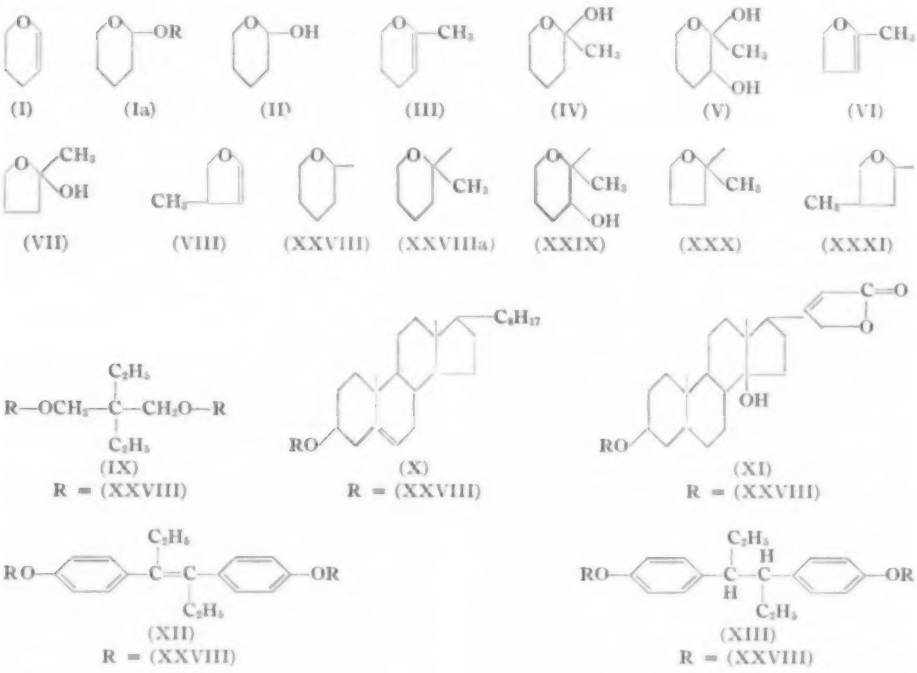
Digitoxigen in (100 mg.) was dissolved in 1 cc. of ethyl acetate followed by the addition of 200 mg. of dihydropyran (I) and one drop of a solution containing one drop of phosphorus oxychloride in 5 cc. of ethyl acetate. This mixture was warmed to 45° for twenty minutes, cooled to room temperature and diluted with 10 cc. of petroleum ether. An amorphous precipitate was obtained that first was crystallized from an ether-petroleum ether mixture, and then from anhydrous ether, and then dried over P_2O_5. M. p., 156–159°. Yield, 66.7%.

Anal.—Calcd. for $C_{29}H_{32}O_5$: C, 73.4; H, 9.3. Found: C, 74.0; H, 9.6.

Anal.—Calcd. for $C_{28}H_{28}O_4$: C, 77.0; H, 8.3. Found: C, 76.6; H, 8.1.

The same product also was obtained when 0.536 Gm. (0.002 mole) of diethylstilbestrol in 2.5 Gm. of dihydropyran (I) and a drop of 5% hydrogen chloride in dioxane were warmed to 55° for 5 minutes and then cooled. Ten cubic centimeters of petroleum ether was added and the mixture cooled to 0° after which the product crystallized.

In another experiment 0.536 Gm. (0.002 mole) of diethylstilbestrol was dissolved in 1.5 Gm. of δ-hydroxyvaleraldehyde (II), followed by the addition of one drop of a 5% solution of hydrogen chloride in dioxane. This mixture was heated in an oil bath at 110° for twenty-five minutes. After cooling the mixture, 10 cc. of 5% aqueous potassium hydroxide was added. The alkaline solution was extracted three times with 20-cc. portions of ether which were combined, washed once with water, the solvent removed, and the residue dried over P_2O_5. Crystallization from ethyl acetate yielded 0.185 Gm. (21.2%) of product. After two more recrystallizations from ethyl acetate the product showed no depression in melting point when mixed with the product obtained from dihydropyran (I) and diethylstilbestrol.



Synthesis of α,α′-Diethyl-4,4′-bis-(2′′-tetrahydropyranyloxy)-stilbene (XII).—Diethylstilbestrol 805 mg. (0.003 mole) was dissolved in 10 cc. of dioxane, to which was added 0.84 Gm. (0.01 mole) of dihydropyran (I) then one drop of a 5% solution of hydrogen chloride in dioxane. This mixture was warmed to 55° for five minutes, then cooled to room temperature followed by the addition of 15 cc. of ether. The resulting ethereal solution was shaken with 10 cc. of 10% aqueous potassium hydroxide to remove the acid and any unreacted phenolic materials. The aqueous alkali was extracted twice with 20-cc. portions of ether and the combined ethereal extracts washed once with 20 cc. of water. The ether was removed and the residue after drying over P_2O_5 was recrystallized twice from ethyl acetate and once from cyclohexane and then dried over P_2O_5. M. p., 185–188°. Yield 54.3%.

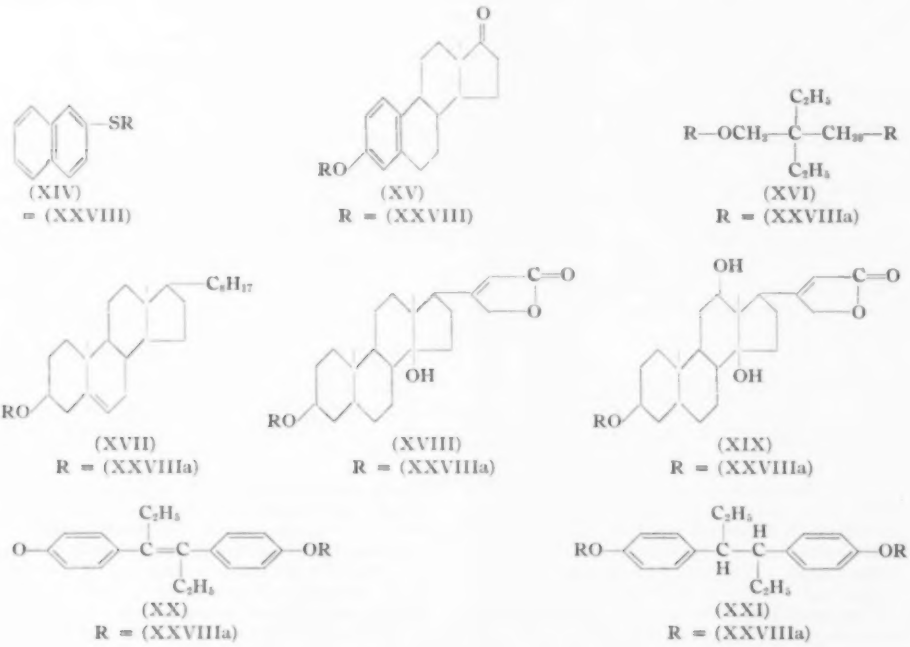
Synthesis of 1,2-Diethyl-4,4′-bis-(2′′-tetrahydropyranyloxy)-1,2-diphenylethane (XIII).—Hexestrol (1 Gm.) was dissolved in 3 cc. of warm dihydropyran (I). On the addition of a drop of phosphorous oxychloride from a capillary, spontaneous heating

occurred and crystallization took place from the warm mixture. The mixture was heated to 60° for five minutes, cooled, and dissolved in 30 cc. of ethyl acetate. On cooling this solution, the product crystallized. M. p., 166–169°. Yield, 88.8%.

Anal.—Calcd. for $C_{28}H_{36}O_4$: C, 76.7; H, 8.7. Found: C, 76.9; H, 9.0.

Synthesis of Naphthalene-2-(2′-tetrahydropyranyl)-sulfide (XIV).—2-Naphthalenethiol[1] 6.4 Gm. (0.04 mole), 3.76 Gm. (0.04 mole) of phenol, and 3.36 Gm. (0.04 mole) of dihydropyran (I) were mixed in a dry 100-cc. flask. Solution occurred on slight warming. Spontaneous heating was obtained when two drops of a 5% solution of hydrogen chloride in dioxane were added and after thirty minutes naphthalenethiol was dissolved in 4 Gm. of δ-hydroxyvaleraldehyde (II) to which one drop of a 5% solution of hydrogen chloride in dioxane was added. The mixture was heated on an oil bath at 120° for forty minutes, then distilled, without neutralizing the acid, to yield the same product as obtained from dihydropyran (I).

Synthesis of 3-(2′-Tetrahydropyranyoxy)-1,3,5-(10)-estratriene-17-one (XV).—Estrone 0.27 Gm. (0.001 mole) and dihydropyran (I) 0.5 Gm. were dissolved in 4 cc. of dioxane, to which was added one drop of a 5% solution of hydrogen chloride in dioxane. This mixture was heated to 50° for thirty minutes and the product obtained in the same manner as was (XII). This product was recrystallized



the mixture was extracted with 60 cc. of 10% aqueous potassium hydroxide. The alkaline aqueous solution was extracted three times with 100-cc. portions of ether and the combined ethereal extracts were washed once with water, concentrated, and dried over P_2O_5. Distillation of the residue yielded a product boiling at 212–217° at 5 mm. Yield, 73.1%. Redistillation three additional times gave a product boiling at 180–183° at 2 mm.

Anal.—Calcd. for $C_{15}H_{16}OS$: C, 73.6; H, 6.6. Found: C, 74.0; H, 6.5.

The above method in which phenol serves as a catalyst is similar to that used by Parham (19). Without the use of phenol, longer periods of time were required.

The same derivative was obtained when the following procedure was employed. Four grams of from ethyl acetate. M. p., 147–148°. Yield, 63.8%.

Anal.—Calcd. for $C_{29}H_{31}O_3$: C, 77.9; H, 8.5. Found: C, 78.2; H, 8.6.

Synthesis of 2,2-Diethyl-1,3-bis-(2′-(2′-methyl)-tetrahydropyranyloxy)-propane (XVI).—This product was prepared from anhydro-δ-aceto *n*-butyl alcohol (III) and from δ-aceto-*n*-butyl alcohol (IV) by the same methods used to synthesize (IX). B. p., 172–178° at 6 mm. Yield, 83.5%.

Anal.—Calcd. for $C_{19}H_{36}O_4$: C, 69.5; H, 11.1. Found: C, 69.8; H, 11.7.

Synthesis of 3β-2′-(2′-Methyl)-tetrahydropyranyloxy Δ^5-cholestene (XVII).—Cholesterol 1.93 Gm. (0.005 mole) was dissolved in 8 cc. of dioxane and 0.74 Gm. (0.0075 mole) of anhydro-δ-aceto-*n*-butyl alcohol (III). A drop of phosphorous oxychloride was added from a capillary, and the mixture warmed to 50° for fifteen minutes. After cooling for about thirty minutes, crystallization of the product from

[1] Eastman Kodak Co.

the reaction mixture took place. Further crystallization occurred at 12°. The product was recrystallized from ethyl acetate and dried over P_2O_5. M.p., 174–177°. Yield, 90.4%.

Anal.—Calcd. for $C_{32}H_{52}O_2$: C, 81.6; H, 11.6. Found: C, 81.6; H, 11.7.

Synthesis of 3β-2′-(2′-Methyl)-tetrahydropyranyloxy-14β-hydroxy-20(22)-cardenolide (XVIII).—Digitoxigenin (0.125 Gm.) was added to 0.75 Gm. of anhydro-δ-aceto-*n*-butyl alcohol (III). The digitoxigenin was not soluble in this quantity of solvent, however, on addition of eight drops of a solution containing one drop of $POCl_3$ in 10 cc. of ethyl acetate, spontaneous warming and solution occurred. The solution was warmed to 60° for five minutes, then allowed to cool slowly, at which time crystallization of the product took place. Further yields were obtained by addition of ether to the mother liquor. The product was recrystallized from cyclohexane.

When a melting point was taken, decomposition occurred, and the residue would melt at 253–254°, which was the same as that for digitoxigenin. However, when the melting point block was preheated to 145°, and the temperature allowed to rise at 10°/minute, the product would melt and decompose at 152–156°. As the temperature was raised, it would resolidify and remelt at 253–254°. Yield, 80%.

Anal.—Calcd. for $C_{29}H_{46}O_5$: C, 73.7; H, 9.4. Found: C, 73.8; H, 9.6.

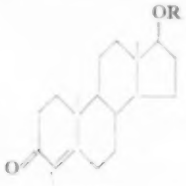
Synthesis of 3β-2′-(2′-Methyl)-tetrahydropyranyloxy-12β, 14β-dihydroxy-20(22)-cardenolide (XIX).—Digoxin[4] (2 Gm.) was hydrolyzed in a manner similar to that used for digitoxin, yielding 0.575 Gm. of digoxigenin, m. p. 217–219°.

Digoxigenin (0.150 Gm.) was added to 1.4 Gm. of anhydro-δ-aceto-*n*-butyl alcohol (III) to which three drops of a solution containing one drop of phosphorous oxychloride in 10 cc. of ethyl acetate was added. The mixture was heated to 65° for ten minutes in order to dissolve the digoxigenin. After cooling the mixture to room temperature, 30 cc. of anhydrous ether was added, and on cooling to 0°, crystallization of the product took place. It was recrystallized twice from anhydrous ether. M. p. 159–163°. Yield, 62%.

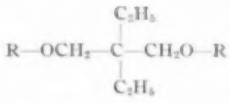
Anal.—Calcd. for $C_{29}H_{46}O_6$: C, 71.3; H, 9.1. Found: C, 71.4; H, 9.1.

It seems apparent that the C-3 hydroxyl was the one which was involved in ketal formation from evidence presented relating to steric hindrance at C-12.

Synthesis of α,α′ -Diethyl-4,4′-bis (2″-(2″-methyl)-tetrahydropyranyloxy)-stilbene (XX).—Diethylstilbestrol was dissolved in 2 cc. of (0.536 Gm., 0.002 mole) anhydro-δ-aceto-*n*-butyl alcohol (III)

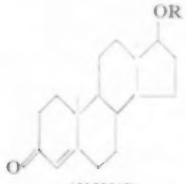


(XXII)
R = (XXVIIIa)



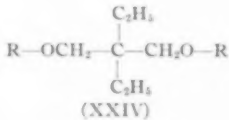
(XXIII)
R = (XXIX)

(XXV)
R = (XXX)



(XXVI)
R = (XXX)

(XXVII)
R = (XXXI)



(XXIV)
R = (XXX)

and the reaction catalyzed by addition of one drop of a 5% solution of hydrogen chloride in dioxane. After thirty minutes at room temperature, the reaction mixture was made alkaline with two drops of 10% ethanolic potassium hydroxide. It was then diluted with 15 cc. of petroleum ether and filtered. After two days at −5° a crystalline product was obtained from the filtrate. This compound was very unstable. Recrystallization was carried out from petroleum ether containing a drop of (III). The melting point was determined by the same technique as employed for (XVIII). When the per cent carbon and hydrogen was determined by conventional methods, the product would decompose and flash burn in the tube and a good analysis was not obtained in this way. A weighed sample of this material was heated in the dry state to 140° for five minutes while a stream of nitrogen was used to carry away the vapors of (III). The odor of (III) as a decomposition product was apparent. The residue was re-weighed and found to check exactly with the calculated weight for diethylstilbestrol. M. p. 114–116°. Yield, 66.8%.

Synthesis of 1,2-Diethyl-4,4′-bis-(2″-(2″-methyl)-tetrahydropyranyloxy) - 1,2 - diphenyl - ethane (XXI).—One Gm. of hexestrol was dissolved in 2 cc. of anhydro-δ-aceto-*n*-butyl alcohol (III) to which one drop of a solution containing one drop of phosphorous oxychloride in 5 cc. of ethyl acetate

[4] Burroughs-Wellome & Co.

was added. The mixture which warmed spontaneously was heated to 55° for ten minutes. The product was isolated in the same way as was (XII), and exhibited signs of unstability. It was recrystallized from petroleum ether, and the melting point determined by the same technique as used for (XX) was 115–116°, yield 63.5%.

Anal.—Calcd. for $C_{26}H_{42}O_4$: C, 77.2; H, 8.8. Found: C, 77.5; H, 9.1.

Synthesis of 17β-2′-(2′-methyl)-tetrahydropyranyloxy-Δ⁴-androstene-3-one (XXII).—Testosterone (0.150 Gm.) was dissolved in 2 cc. of chloroform and 0.5 Gm. of anhydro-δ-aceto-*n*-butyl alcohol (III). A drop of phosphorous oxychloride was added from a capillary and spontaneous heating occurred. After thirty minutes at room temperature 10 cc. of petroleum ether was added and the mixture cooled to −5°. Crystallization occurred when the walls of the container were scratched with a glass stirring rod. The product was recrystallized from ethyl acetate. M. p. 139–142°. Yield, 79%.

Anal.—Calcd. for $C_{25}H_{37}O_2$: C, 77.7; H, 9.9. Found: C, 77.4; H, 9.4.

Synthesis of 2,2-Diethyl-1,3-bis-(2′-(2′-methyl-3′-hydroxy)-tetrahydropyranyloxy)-propane (XXIII).—Prenderol 0.66 Gm. (0.005 mole) was mixed with 1.32 Gm. (0.01 mole) of 3-hydroxy-δ-aceto-*n*-butyl alcohol (V) and warmed on a hot plate until the mixture liquefied. After cooling to 40°, a drop of phosphorous oxychloride was added from a capillary and spontaneous heating occurred, after which external heat was applied to bring the temperature to 80°. After a few minutes crystallization began in the hot mixture. Heating was continued an additional twenty minutes before cooling to room temperature at which point crystals were obtained. Addition of petroleum ether to the filtrate induced further crystallization of more product.

The product thus obtained sublimed at temperatures above 125°, and was purified by sublimation at atmospheric pressure. The melting point was taken in a sealed capillary in an oil bath. M. p. 211–214°. Yield, 59%.

Anal.—Calcd. for $C_{18}H_{36}O_6$: C, 63.3; H, 10.1. Found: C, 63.3; H, 9.8.

Smaller yields of this product were obtained by using ethyl acetate as a solvent for the reaction mixture.

Synthesis of 2,2-Diethyl-1,3-bis[2′-(2′-methyl)-tetrahydrofuranyloxy]-propane (XXIV).—This product was obtained from the reaction of Prenderol[8] with anhydro-γ-aceto-*n*-propyl alcohol (VI) and with γ-aceto-*n*-propyl alcohol (VII) in the same manner as employed for (IX).

Synthesis of 3β-2′-(2′-Methyl)-tetrahydrofuranyloxy Δ⁵-cholestene (XXV).—One Gm. of cholesterol was dissolved in 2.5 cc. of warm anhydro-γ-aceto-*n*-propyl alcohol (VI) and the reaction was catalyzed with one drop of a 5% solution of hydrogen chloride in dioxane. After warming to 55° for five minutes, the mixture was allowed to cool, at which time crystallization of the product took place. It was recrystallized from ethyl acetate and then from petroleum ether. M. p. 131–135°, yield, 83.3%. A mixed melting point with cholesterol depressed the melting point to 115–117°.

Anal.—Calcd. for $C_{33}H_{57}O_2$; C, 81.7; H, 11.6. Found: C, 82.2; H. 12.3.

Synthesis of 17β-2′-(2′-Methyl)-tetrahydrofuranyloxy-Δ⁴-androstene-3-one (XXVI).—Testosterone (0.125 Gm.) was added to 0.6 cc. of anhydro-γ-aceto-*n*-propyl alcohol (VI). Five drops of a solution containing one drop of phosphorous oxychloride in 5 cc. of ethyl acetate were added, while the contents of the reaction flask were kept cool in a cold water bath. The testosterone was not soluble in (VI) until addition of the $POCl_3$, after which it dissolved and the product soon began to crystallize. The addition of 2 cc. of ethyl acetate caused the product to redissolve, and the solution was heated to 50° for five minutes. On cooling, crystallization of the product occurred. Recrystallization was accomplished by dissolving in ethyl acetate at room temperature, then cooling to 0°. When a melting point was taken, the plate was preheated to 125°, M. p. 132—135°, yield 74%.

Synthesis of 3β-2′-(4′-Methyl)-tetrahydrofuranyloxy Δ⁵-cholestene (XXVII).—Cholesterol 0.96 Gm. (0.0025 mole) was dissolved in 4 cc. of dioxane to which 0.42 Gm. (0.005 mole) of (VIII) and a drop of $POCl_3$ from a capillary were added. After heating to 50° for five minutes the mixture was allowed to cool slowly, whereupon the product crystallized out.

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Antispasmodics IV. Further Basic Alkyl Ester Acid Addition and Quaternary Ammonium Salts of Alpha-(2-Cycloalkenyl)-2-thienylacetic Acids*

By FREEMAN H. McMILLAN and CHARLES C. SCOTT

The β-diisopropylaminoethyl esters of α-(2-cyclopentenyl)-α-(2-thienyl)-acetic acid and of α-(2-cyclohexenyl)-α-(2-thienyl)-acetic acid have been prepared as well as their hydrochlorides and methobromides which have been found to have appreciable antispasmodic activity. In addition the 1 methyl-4-piperidyl esters of both the above named acids have been converted to a variety of quaternary ammonium salts by treatment with a series of alkyl and aralkyl halides in order to ascertain the effect on antispasmodic activity of varying both the organic cation and the inorganic anion of such quaternary salts.

SERIES OF basic alkyl esters of α-(2-cyclopentenyl)-α-(2-thienyl)acetic and α-(2-cyclohexenyl)-α-(2-thienyl)-acetic acids have been previously reported from these laboratories (1) together with a brief statement of the antispasmodic activity of their acid addition salts and their quaternary ammonium salts. The use of β-diisopropylaminoethanol to prepare potent antispasmodic compounds has been reported (2–4) and it was decided to prepare esters of our previously reported acids (1) with this alcohol. Each of these esters was prepared by treatment of the alcohol with the appropriate acid chloride (1) and then converted to its hydrochloride and its methobromide quaternary salt.

Since the methiodides of 1-methyl-4-piperidyl α-(2-cyclopentenyl)-α-(2-thienyl)-acetate and 1-methyl-4-piperidyl-α-(2-cyclohexenyl)-α-(2-thienyl)-acetate (Compound 35 of Table III and Compound 25 of Table IV, respectively, of reference 1) were found to possess particularly interesting antispasmodic activity it was decided to replace the methyl iodide quaternizing agent used to prepare the above compounds with a series of alkyl and aralkyl halides in order to study the effect of varying both the size of the organic cation and the inorganic anion on the antispasmodic activity of a typical fairly active compound. These quaternary salts were prepared by mixing the basic ester with the requisite alkyl or aralkyl halide in acetone at a temperature varying with the reactivity of each particular halide.

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The authors are indebted to Charles M. Anderson, Jerome D. Genzer, Kenneth A. Kun, and Carol B. McMillan for assistance in the preparation of some of the compounds. M. Chessin and W. E. Rowe rendered valuable aid in the pharmacologic studies.

PHARMACOLOGY

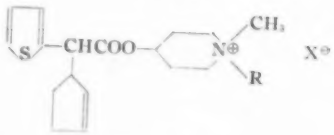
Methods.—In most instances the antispasmodic activity of these compounds was tested both on isolated smooth muscle and in intact animals. For the former, the standard Magnus method was used. In the latter case, the compounds were assayed by intravenous injection into anesthetized cats. To accomplish this a rubber balloon one inch long (finger-cot) was attached to a catheter. Following a midline incision the balloon was inserted into the duodenum through a small incision in the gut. The midline incision was then sutured. The catheter was connected to a sensitive tambour recording kymographically. The balloon was inflated with only 1.5 cc. of air. Such a system showed spontaneous motility which was regular and continuous. The contractions are apparently of the segmental type. The potency of the various compounds was determined by comparing the minimal dose necessary to produce definite inhibition of motility for three to five minutes.

Four of the compounds were compared with atropine for antispasmodic activity in unanesthetized dogs. These animals had permanent fistulas in the gastrointestinal canal made by insertion of stainless steel cannulas. After the animals were trained to lie quietly on a table, a balloon similar to that used in cats mentioned above was introduced through the fistula into a part of the small bowel. During control periods continuous segmental contractions were seen after the balloon was inflated and connected to a tambour. The antispasmodic agent was administered directly into the gastrointestinal tract either through the recording fistula or else a fistula in the stomach to simulate oral dosing. The dose necessary to produce a definite and marked inhibition of motility lasting for about thirty to sixty minutes was determined in order to compare relative potencies of the compounds.

For about half of the compounds the median lethal dose in mice was determined by both oral and intravenous routes.

Results.—The relative potencies of the compounds by the isolated gut and cat methods are fairly consistent. The procedure in dogs while consistent for the synthetic substances gave a marked difference with atropine. Whereas a number of

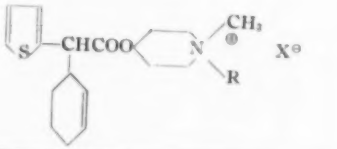
TABLE I



No.	R	X	Empirical Formulas	Reaction Temp., °C.	Yield, %	Recryst. Solvent	M. p., °C.	Analyses, % Calculated C	H	N	X	Found C	H	N	X
1	CH_3	Br	$C_{18}H_{20}BrNO_2S$	30	85	Ethanol-ether	207 –208.5	53.99	6.55	...	19.96	54.10	6.69	...	20.03
2	CH_3	Cl	$C_{18}H_{20}ClNO_2S$	30	77	Ethanol-ether	214 –214.5	60.75	7.36	...	9.96	60.80	7.34	...	10.05
3	CH_3	CH_3SO_4	$C_{19}H_{23}NO_6S_2$	30	67	Acetone	134 –135	52.88	6.77	3.25	...	52.66	6.98	3.43	...
4	C_2H_5	Br	$C_{19}H_{22}BrNO_2S$	80	70	Acetone-ethanol	173.5–175	55.07	6.81	...	19.29	54.84	6.94	...	19.21
5	Allyl	Br	$C_{20}H_{22}BrNO_2S$	60	51	Acetone-ethanol	140 –141.5	56.33	6.62	...	18.74	56.25	6.78	...	18.76
6	i-C_3H_7	I	$C_{20}H_{30}INO_2S$	80	16	Acetone	148 –150	...	...	2.95	26.70	...	...	2.84	26.45
7	$C_6H_5CH_2$	Br	$C_{24}H_{24}BrNO_2S$	80	24	Acetone	162 –165	...	...	2.94	16.77	...	...	2.91	16.53
8	p-$ClC_6H_4CH_2$	Br	$C_{24}H_{23}ClBrNO_2S$	60	25	Acetone	170 –171	56.42	5.72	...	15.64[a]	56.21	5.90	...	15.58[a]

[a] This is a bromide analysis.

TABLE II



No.	R	X	Empirical Formulas	Reaction Temp., °C.	Yield, %	Recryst. Solvent	M. p., °C.	Analyses, % Calculated C	H	N	X	Found C	H	N	X
9	CH_3	Br	$C_{19}H_{22}BrNO_2S$	30	82	Ethanol-ether	192.5–193.5	55.07	6.81	...	19.29	55.05	6.93	...	19.31
10	CH_3	Cl	$C_{19}H_{22}ClNO_2S$	30	40	Ethanol-ether	208	61.68	7.63	...	9.59	61.57	7.84	...	9.60
11	CH_3	CH_3SO_4	$C_{20}H_{25}NO_6S_2$	30	37	Ethanol-acetone	138 –140	53.91	7.01	3.14	...	54.06	7.11	3.08	...
12	C_2H_5	Br	$C_{20}H_{24}BrNO_2S$	80	78	Ethanol-ether	209 –210	56.07	7.06	...	18.65	55.78	7.00	...	18.72
13	Allyl	Br	$C_{21}H_{24}BrNO_2S$	60	55	Acetone	125 –127	...	...	3.25	18.15	...	...	3.16	17.85
14	i-C_3H_7	I	$C_{21}H_{32}INO_2S$	80	25	Acetone	169 –171	51.52	6.59	...	25.9	51.80	6.73	...	25.9
15	$C_6H_5CH_2$	Br	$C_{25}H_{26}BrNO_2S$	80	85	Isopropanol	159.5–161.5	61.21	6.58	...	16.29	60.71	6.68	...	16.25
16	p-$ClC_6H_4CH_2$	Br	$C_{25}H_{21}BrClNO_2S$	60	45	Isopropanol	183 –184	57.20	5.95	...	15.22[a]	57.22	6.22	...	15.35[a]

[a] This is a bromide analysis.

compounds were considerably more potent than atropine *in vitro* and *in vivo* in cats, they were less active by oral administration to dogs. This reflects the well known fact that atropine is more potent than most synthetic antispasmodics in human beings receiving the drugs orally. Results are shown in Table III.

There seemed little and no consistent difference between the compounds containing the cyclopentenyl or cyclohexenyl rings. Regarding alkyl or aralkyl substitution on the N-atom, the decreasing order of potency was as follows: methyl > ethyl > allyl > isopropyl > benzyl > *p*-chlorobenzyl. The acid radical also influenced antispasmodic activity in the following order: chloride > bromide > methosulfate. Concerning acute toxicity, it is noteworthy that the oral toxicities of the quaternary ammonium compounds are about $^1/_{100}$ the intravenous value. In the case of II-hydrochloride and atropine, this ratio is only $^1/_{10}$ to $^1/_{20}$. This probably indicates better absorbability of these tertiary amines.

tion of α-(2-cyclopentenyl)-α-(2-thienyl)-acetyl chloride in 50 ml. of dry benzene. When the addition was complete the mixture was heated at reflux for fifteen minutes. Triethylamine hydrochloride was filtered from the cooled reaction mixture and the benzene was removed from the filtrate by distillation under vacuum. The residue was a viscous oil which boiled at 138–142° (0.3 mm.). Yield, 30.0 Gm. (86% of theory).

I-Hydrochloride.—The above base (50.3 Gm., 0.15 mole) was dissolved in 250 ml. of ethyl acetate and dry hydrogen chloride was bubbled into the solution until it was acid to methyl red. The cooled solution yielded the crystalline hydrochloride. Yield 45.6 Gm. (84% of theory) melting at 136–138°. A small sample recrystallized from ethyl acetate melted at 138–140°.

Anal.—Calcd. for $C_{19}H_{30}ClNO_2S$: N, 3.77; Cl, 9.46. Found: N, 3.67; Cl, 9.53.

I-Methobromide.—The above base (50.3 Gm., 0.15 mole) dissolved in 75 ml. of acetone was mixed with a solution of methyl bromide (14.3 Gm.,

TABLE III

Compound	LD_{50}, mg./Kg. I. v.	LD_{50}, mg./Kg. P. o.	Antispasmodic Activity: Isolated Guinea Pig Ileum, mcg.[a]	Antispasmodic Activity: Anesthetized Cat, Relative Potency, I. v.	Antispasmodic Activity: Trained Dog, Dose to Inhibit, mg./Kg., P. o.
Atropine sulfate	86.0	800	0.01	1.0	0.2–0.8
10	3.9	630	0.001–0.005	10.5	1 –2
11	5.15	470	0.001–0.005	4.6	
1	6.1	596	0.013–0.025	8.0	2 –5
I-Methobromide	...	...	>1.0	2.8	...
3	4.38	817	0.01 –0.05	2.2	
9	...	...	1.0	6.0	2 –5
I-Hydrochloride	...	...	0.1	0.5	...
8	...	...	1.0	0.08	...
II-Hydrochloride	62.8	1450	0.01 –0.1	0.36	15
II-Methobromide	4.4	340	0.005–0.01	2.17	...
2	5.0	525	0.001–0.01	13.3	...
5	4.3	390	0.001–0.01	1.8	...
15	...	...	0.1 –1.0	0.24	...
4	...	...	0.01 –0.1	4.55	...
16	...	...	1.0	0.07	...
12	...	...	...	3.5	...
14	...	...	...	1.4	...
7	...	...	...	2.4	...
13	...	...	0.1 –1.0	4.4	...

[a] Concentration of drug in mcg./ml. of bath needed to inhibit stimulating effect of acetylcholine chloride 1 mcg./ml. of bath.

EXPERIMENTAL[1]

β-Diisopropylaminoethyl-α-(2-cyclopentenyl)-α-(2-thienyl)-acetate (I).—In a three-necked round-bottomed flask fitted with a stirrer, a thermometer, a reflux condenser with a drying tube, and a dropping funnel there was placed β-diisopropylaminoethanol (14.5 Gm., 0.10 mole) and triethylamine (12 Gm., 0.10+ mole) and 150 ml. of dry benzene; with cooling to 10° there was slowly added a solu-

[1] All melting points and boiling points are uncorrected. Microanalyses were carried out by Dr. F. Buhler and Miss Lorelinde Einstein.

0.15 mole) in 150 ml. of acetone; the mixture was heated in a pressure bottle at 80° for twenty-four hours. The cooled mixture yielded 20 Gm. (31% yield) of crystals melting at 155–157°. One recrystallization from ethanol-ether gave material melting at 156–158°.

Anal.—Calcd. for $C_{20}H_{32}BrNO_2S$: N, 3.25; Br, 18.29. Found: N, 3.17; Br, 18.57.

β-Diisopropylaminoethyl α-(2-cyclohexenyl)-α-(2-thienyl)-acetate (II).—By the same procedure used to prepare I this compound was prepared in 86% yield boiling at 143–146° (0.3 mm.).

II-Hydrochloride.—By the procedure used to pre-

pare I-hydrochloride, II-hydrochloride was obtained in 78% yield melting at 119–121°.

Anal.—Calcd. for $C_{20}H_{32}ClNO_2S$: N, 3.63; Cl, 9.18. Found: N, 3.46; Cl, 8.89.

II-Methobromide.—By the procedure used to prepare I-methobromide, II-methobromide was obtained in 53% yield melting at 176–178°.

Anal.—Calcd. for $C_{21}H_{34}BrNO_2S$: N, 3.15; Br, 17.98. Found: N, 3.08; Br, 18.05.

Reactions of 1-Methyl-4-piperidyl Esters with Alkyl and Aralkyl Halides.—These reactions were all carried out by mixing the basic ester with the requisite halide in acetone and heating the mixture at a temperature necessary to enable reaction to take place. Table I lists the quaternary salts derived from 1-methyl-4-piperidyl α-(2-cyclopentenyl)-α-(2-thienyl)-acetate together with necessary data concerning their preparation, properties, and analyses. Table II lists the same data for the quaternary salts derived from 1-methyl-4-piperidyl α-(2-cyclohexenyl)-α-(2-thienyl)-acetate.

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A Note on Tigogenin from *Digitalis Lanata**

By LEMONT KIER and OLE GISVOLD

DURING the phytochemical investigations of the fresh leaves of *Digitalis lanata* for its cardiac glycosides, large quantities of aqueous extracts were made available. Previous investigations (1) had shown that such aqueous extracts from other Digitalis extracts contained saponins that could be isolated in a fair state of purity. By following these techniques a considerable quantity of a crude saponin mixture was obtained. At the time of this investigation no particular interest was manifested on our part as to the exact nature of the saponins *per se* by virtue of the difficulties that might be experienced in their purifications. Studies on the nature of the sapogenin were therefore initiated.

EXPERIMENTAL

Isolation of the Crude Saponins.—The isolation and partial purifications of the saponins from *Digitalis lanata* were carried out essentially according to the techniques described by Appel and Gisvold (1).

Hydrolysis of Saponin.—The less butanol-soluble saponin was dissolved in 20 cc. of ethanol and 40 cc. of water to which 3.44 cc. of concentrated sulfuric acid and 5 cc. of *n*-amyl alcohol was added. The mixture was refluxed for two and one-half hours. Upon cooling a white solid separated that was crystallized twice from methanol and once from acetone, m. p. 196–198°. Reported (2) m. p. for tigogenin is 203–204°.

Sapogenin (Tigogenin) Acetate.—Although the acetate of tigogenin is not very satisfactory because of its melting point, it was prepared for infrared studies. The acetate prepared in the usual way from acetic anhydride and sodium acetate was recrystallized twice from ethanol, m. p. 194–196°. Mixed melt with the original sapogenin was 174–177°.

Homogeneity of the Sapogenin.—The application of paper chromatography was used to establish the homogeneity of the sapogenin. Propylene glycol was used as the stationary phase on Whatman No. I paper. Benzene-chloroform (9:1) was used as the mobile phase with a running time of sixteen hours. The paper after drying was sprayed with a 25 per cent solution of trichloroacetic acid in chloroform. It was then heated for five minutes at 90° by means of a battery (four) of infrared lamps. When examined under an ultraviolet lamp, only one fluorescent spot was observed.

Infrared Studies.—A sample of the sapogenin and its acetate were examined for their infrared spectra through the courtesy of Dr. R. Norman Jones of the National Research Council, Canada. The spectra show a very close resemblance to the curves for tigogenin (3) and tigogenin acetate (3)

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* Received April 6, 1956, from the College of Pharmacy, University of Minnesota, Minneapolis.

Book Notices

The Flavonoids in Biology and Medicine. By MAURICE E. SHILS and ROBERT S. GOODHART. The National Vitamin Foundation, Inc., New York, 1956. iii + 101 pp. 15 x 23 cm. Price $2.

This second volume in a Nutrition Monograph Series is a critical review of laboratory and clinical reports on the actions and therapeutic applications of flavonoids. Their review of the material which includes 299 references to published reports leads the authors to conclude that there is no convincing evidence for a nutritional role of flavonoids, and that in claiming therapeutic value for them in the treatment of disorders of the vascular system the burden of proof of validity rests with the claimants. The authors recommend that adequate clinical studies should be conducted to clarify the actions and uses of flavonoids.

Synthetic Drugs. By H. RONALD FLECK. Elsevier Press, Inc., New York, 1955. viii + 380 pp. 15.5 × 26 cm. Price $12.50.

This British book represents an attempt to collate the synthetic, analytical, and clinical aspects of synthetic drugs. The drugs are divided according to therapeutic classification and are grouped by related molecular structures. The information for each drug is arranged in the form of a brief monograph giving: systematic (chemical) name, structural formula, synthesis, properties, analysis and testing, clinical data, and dosage. Although the author presents the material in very abbreviated form, references to original literature are given and important points are included. Each chapter has an introduction to the therapeutic class of compounds. Certain compounds are included to illustrate their particular types of syntheses. A supplement discusses the commercial synthesis of chloramphenicol, the properties of carbomycin, erythromycin, neomycin, tetracycline, viomycin, and the recent antispasmodic compound cyclospasmol.

The book has a general index and a clinical index which add to the usefulness of the text as a reference aid. The book should be particularly useful to pharmacists and pharmaceutical chemists.

Kommentar zur Pharmacopoea Helvetica. 5th ed. Supplements I and II. By PAUL CASPARIS, JAKOB BÜCHI, HANS FLÜCK, ERNST KÜHNI, KURT MÜNZEL, and ROBERT REGAMEY. xii + 316 pp. 17.6 x 21.1 cm. Price fr. 32.

The commentary (in German) on Ph. H. V, Supplement I, contains information relating to one hundred and twenty-two new monographs, with sections on antigens, tablets, and serums. Much useful information is included in the material arranged in monograph style. Particularly interesting are the condensed discussions on synthesis and reactions of some chemical medicinals. The commentary on Ph. H. V, Supplement II, includes sections on sterilization, optical instrumentation, and vitamin preparations. Changes and additions in monographs and tables in Ph. H. V are indicated. A list of test reagent chemicals, with the material for which the reagent is used, is included. The Commentary on both Supplements is beautifully prepared and bound and a cumulative index for the Commentary on Ph. H. V, the Addendum to the Commentary, and the present Commentary on the Supplements is appended.

Alcoholism—Its Psychology and Cure. By FREDERICK B. REA. The Philosophical Library, Inc., New York, 1956. 143 pp. 12.5 x 19 cm. Price $3.50.

This book presents a limited review of the causes and treatment of alcoholism. It attempts to explain the physical, psychological, and spiritual factors involved in this problem of social reform and public health, and discusses the ways of dealing with these factors.

Teeanalyse. By LUDWIG HÖRHAMMER. Institut für Pharmazeutische Arzneimittellehre der Universität München, 1955. 75 pp. 556 illustrations. 22 x 31 cm. Price DM 58.

This German text discusses the various drugs that have been found as constituents and adulterants in teas and tea blends. The microscopic and macroscopic characteristics are described and comparisons of similar drugs are made. Components and pharmaceutical action of herbal blends are given. The 275 drugs include 30 leaves, 77 tops, 34 flowers, 24 fruits, 8 seeds, 7 woods, 25 barks, 36 roots, 12 rhizomes, and 22 miscellaneous drugs. Accompanying the text, but on separate sheets, are 556 photographic illustrations of the drugs discussed. The "atlas" should be of interest to pharmaceutical libraries as well as to specialists in the pharmacognosy of teas.

Exposés Annuels de Biochimie Médicale. By H. BERNARD, *et al.* Masson et Cie, Paris, 1955. 348 pp. 16.5 x 25.5 cm. Price 3,700 fr.

This is the seventeenth annual compilation (in French) of reports related to medical biochemistry. The subjects presented in this volume are: some aspects of the biochemistry of hemoglobinogenesis, end products of C^{14} labeled glucose and fructose in isolated tissues, synthesis of fats in adipose tissues, some aspects of the study of the metabolism of coenzymes in animals, thyroid hormones, metabolism of diaminated acids, the seromucoids of blood—

their relation to the mucoproteins of the fundamental substance of connective tissue, new data on the biosynthesis of enzymes—enzymatic adaptation, some physicochemical and physiopathological aspects of serum cholesterol, recent biochemical data on the primary diseases of muscle, melanogenesis, the role of lipides in enzymatic systems. The last report is by J. Polonovski (1889–1954) who started this series and to whose memory this volume is dedicated. A cumulative authors index for volumes I to XVII is appended.

Očkování Proti Tuberkulose (*Vaccination Against Tuberculosis*). Collection of Communications presented at the International Congress of the Pneumological and Phthisiological Society in Prague. State Health Institute Library, Prague, 1955. 282 pp. 17 x 24.5 cm.

This book is a compilation of thirty-three reports, twenty-eight of which concern BCG vaccination and five others report experiences with vaccine prepared from *Mycobacterium tuberculosis* typus Murinus-Wells. The papers are in the Czech language with summaries in Russian and English. BCG vaccination is regarded highly, and one part of the final resolution of the Congress states: "It is necessary to give anti-tuberculosis vaccination first phase among all preventive measures, and to ensure for it priority among all the other tasks of the Public Health Institute in the fight against tuberculosis."

Le Cancer. By J. RABINSON. Vigot Frères, Éditeurs, Paris, 1956. 117 pp. 14.5 x 23 cm.

This small paper-back book (in French) considers cancer as a disease which exhausts the neuro-endocrine centers. The text is divided under the following chapter headings: the value of a hypothesis, precancerous states, pathogenic reflections in connection with a second case of cancer in man, heredity and cancer, diagnosis of cancer, cancerous and anticancerous states, cancer an avoidable disease, physiopathologic hypothesis of the neoplastic potentiality, cancer and existence. No references are given and no index is included.

Contribution clinique a l'étude du Métabolisme du Cholestérol. By HENRI QUILLIOT. Vigot Frères, Editeurs, Paris, 1956. 104 pp. 16 x 24.5 cm. Price 450 fr.

In this condensed and illustrated book (in French), the author discusses part of his studies on cholesterol. He associates cholesterol with the functions of digestion, organic tissues, and hormones. The text material is presented under the following chapter headings: composition and origin of cholesterol, physiology of cholesterol, cholesterol and sex hormones, cholesterol and cortico-suprarenal hormones, cholesterol and thyroid, diseases of cellular permeability, cholesterol and diabetes, conclusions, and treatment of hypercholesterolemias. More than half the text is devoted to the relation of cholesterol to diabetes, and in an appendix the author discusses his views regarding the place of acidosis in an organic tissue theory of diabetes. A list of authors consulted is given but no author or subject index is included.

Electrophorèse sur Papier. By CH. WUNDERLY. Vigot Frères, Editeurs, Paris, 1956. 132 pp. 16 x 24.5 cm. Price 650 fr.

This is a translation into French, by Jana Gregoire and Jacques Reynaud, of the book (in German) by Ch. Wunderly. The author attempts to indicate the possibilities of electrophoretic technique and to interest the reader in utilizing the method. Much of the text is devoted to problems which can be managed successfully by electrophoresis. The subject is presented with a brief discussion of the history and terminology followed by the three main divisions under the headings: theoretical bases, methods, and results. References are given throughout the text. The printing and paper are good, although the book has a paper cover. The figures and pictures are clear. The appended author and analytical indexes should be helpful to those who wish to use this book rather than the original German text. An English translation should be well received.

Therapy of Fungus Diseases. Edited by THOMAS H. STERNBERG and VICTOR D. NEWCOMER. Little, Brown and Company, Boston. xxiii + 337 pp. 15.5 x 24.5 cm. Price $7.50.

This book is a compilation of fifty-four papers that were presented at an international symposium on the extent and importance of fungus infections and therapeutic measures against them. The symposium, arranged by the Medical School of the University of California at Los Angeles, provides an up-to-date review and summary of present knowledge of this subject. New material is presented on the recently introduced antifungal antibiotic nystatin (Mycostatin) and other antifungal agents including: diphenylpyraline, undecylenic acid, sulfur, aromatic diamidines, cinnamic acids, nitrostyrenes, filipin, sulfonamides, sulfones, chlorquinaldol, 1968, actidione, stilbamidines, MRD-112, rhodanine, and others. Biological aspects of fungus infections are also discussed from many points of view.

Indexes of contributors and subjects are appended. The book should be a useful addition to all medical and pharmaceutical libraries.

Dictionary of Dietetics. By RHODA ELLIS. Philosophical Library, Inc., New York, 1956. 152 pp. 14 x 21 cm. Price $6.

This is a compilation of terms and references related to diet and diet therapy. Definitions are clear and concise. Cross references are few and one finds "ascorbic acid," "cyanocobalamin" and "pyridoxine," but not under the heading "vitamins." It is up to date. Cold sterilization is defined as "A method whereby electron and X-ray are used to preserve food rather than the use of heat in an attempt to conserve food nutrients which would ordinarily be destroyed by heat." It is a good reference book for dietitians, nutritionists, and nurses.

Handbook of Poisons. By ROBERT H. DREISBACH. Lange Medical Publications, California, 1955. 426 pp. 10 x 18 cm. Price $3.

This book is a concise summary of the diagnosis and treatment of clinically important poisons. The need for such a book has grown with the introduction of many commercial preparations which do not indicate the common name of the toxic ingredient. The text is introduced with a section on general considerations of poisons and poisoning including procedures used in the treatment of acute poisoning. This is followed by sections in which poisons are divided under the headings: pesticides and other agricultural poisons, industrial hazards, household chemicals, medicinal poisons, plant and animal hazards. The section on medicinal poisons occupies 116 pages of the book, and the materials are treated in groups according to their therapeutic usage. Clinical findings to aid in diagnosis are given after each group of substances having similar toxic reactions. Methods of treatment are given in concise form immediately thereafter.

A general index, a list of useful drugs and equipment, and a brief outline of first-aid measures are appended for ready reference. The book is written for the physician, but the pharmacist will find it a handy reference for information the physician might request of him.

Medical Parasitology. By WILLIAM G. SAWITZ. McGraw-Hill Book Company, Inc., New York, 1956. ix + 342 pp. 15.5 x 23.5 cm. Price $6.

This book, in its second edition, is intended for the use of medical students and practicing physicians. The text is introduced with definitions of terms and a classification of parasites, followed by the major sections under protozoa, helminths, arthropoda, treatment, and techniques for laboratory diagnosis. The chapter on treatment should be of interest to pharmacists. Under each type of infestation, the useful drug or drugs are given, with the description and dosage form, dosage, efficacy, toxicity, and contraindications cited. The text ends with a vocabulary of technical terms, and an index is appended. The book should be available to medical and pharmacy students.

Plant Physiology. 4th ed. By MEIRION THOMAS. Philosophical Library, Inc., New York, 1956. xii + 692 pp. 15 x 22 cm. Price $12.

This fourth edition of *Plant Physiology* is the first revision of the text since 1947. The result is a more extensive treatment incorporating the many advances since the third edition. The most extensive changes will be found in the chapters dealing with enzymes, respiration, and photosynthesis, and in scattered sections dealing with the absorption of water and solutes, transpiration, stomatal movement, Crassulacean acid metabolism, cell growth, and auxins. A completely new chapter reports facts and views about photoperiodism and vernalization and an appendix has been added describing those techniques used in partition chromatography and in experiments with isotopic tracers which have led to some of the developments reported in the main body of the book. The book is particularly valuable as a reference for pharmaceutical chemists interested in this field. A bibliography including 748 references, an author index, and a subject index are appended.

BOOKS RECEIVED

The Annual Register of Pharmaceutical Chemists Published under the direction of the Pharmaceutical Society of Great Britain, 1956. The Pharmaceutical Press, 17 Bloomsbury Square London, W. C. 1. v + 720 pp. 17.5 x 24.5 cm. Price three guineas.

The Flavonoids in Biology and Medicine. By MAURICE E. SHILS and ROBERT S. GOODHART. The National Vitamin Foundation, Inc., New York, 1956. iii + 101 pp.. 15 x 23 cm. Price $2.

Acridines. The Chemistry of Heterocyclic Compounds, Vol. IX. By R. M. ACHESON. Interscience Publishers, Inc., New York xii + 409 pp. 15.5 x 23.5 cm. Price $12.50.

New and Nonofficial Remedies 1956. Evaluated by the Council on Pharmacy and Chemistry. J. B. Lippincott Company, Philadelphia, Pa. xxv + 540 pp. 13 x 19.5 cm. Price $3.35.

Kommentar zur Pharmacopoea Helvetica. 5th ed. Supplements I and II. By PAUL CASPARIS, JAKOB BÜCHI, HANS FLÜCK, ERNST KÜHNI, KURT MÜNZEL, and ROBERT REGAMEY. xii + 316 pp. 17.6 x 21.1 cm. Price Fr. 32.

Modern Chemical Processes. Vol. 4. By the editors of Industrial and Engineering Chemistry. Reinhold Publishing Corporation, New York, 1956. v + 202 pp. 21 x 29 cm. Price $5.

Medicinal Chemistry. Vol. II. By F. F. BLICKE and C. M. SUTER. John Wiley & Sons, Inc., New York, 1956. vi + 311 pp. 15 x 23.5 cm. Price $10.

Introductory Organic Chemistry. 3rd ed. By E. WERTHEIM and HAROLD JESKEY. McGraw-Hill Book Company, Inc., New York, 1956. vii + 476 pp. 16 x 23.5 cm. Price $5.50.

Advances in Enzymology. Vol. 17. Edited by F. F. NORD. Interscience Publishers, Inc., New York, 1956. v + 556 pp. 16 x 24 cm. Price $11.

Textbook of Organic Medicinal and Pharmaceutical Chemistry. 3rd ed. By CHARLES O. WILSON and OLE GISVOLD. J. B. Lippincott Company, Philadelphia, Pa., 1956. xvi + 823 pp. 17.5 x 26 cm. Price $11.

Organic Chemistry. 3rd ed. By LOUIS F. FIESER and MARY FIESER. Reinhold Publishing Corporation, New York, 1956. v + 1,112 pp. 15.5 x 24 cm. Price $10.

Traité de Pharmacie chimique. 4th ed. Vol. I–V. Masson et Cie, Paris, 1956. 4,984 pp. 17 x 25 cm. Price 32,000 fr.

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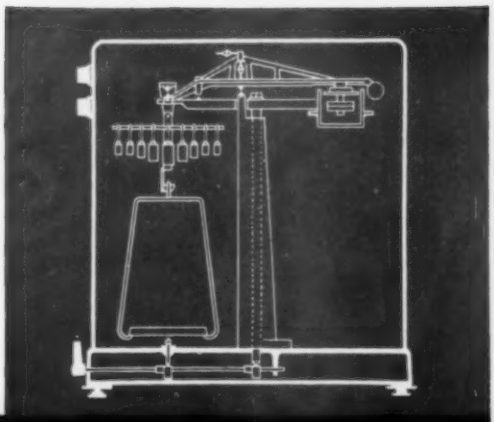

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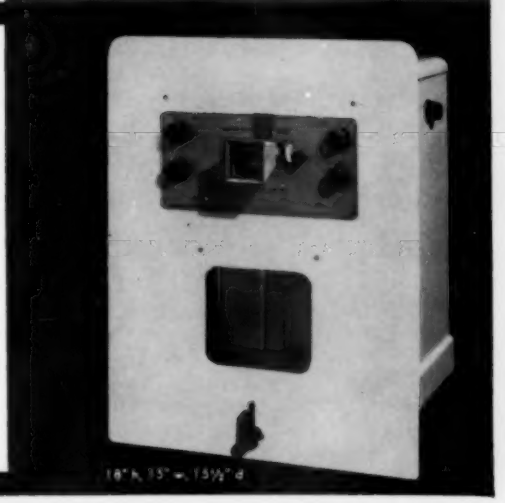

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